Bacterial protein meal as protein source for monogastric animals comparative studies on protein and energy metabolism

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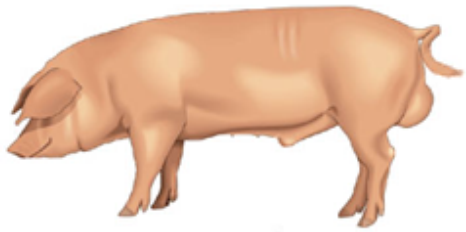
mink



pig

chick

Ph.D. thesis
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Drawings of animals on the front are taken from the *Visual English Dictionary*. QA International Ltd, 2002. *Oxford Reference Online*. Oxford University Press.

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Contents

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Abstract

Bacterial protein meal (BPM) is a new protein feedstuff produced by fermentation of natural gas as energy and carbon source and ammonia as nitrogen source by *Methylococcus capsulatus* (Bath) (>90%), *Ralstonia* sp., *Brevibacillus agri* and *Aneurinibacillus* sp. The bacterial biomass is heat sterilised and spray-dried to obtain a dry and stable product.

The aim of this thesis is to evaluate the effect of increasing dietary level of BPM on the protein and energy metabolism in the growing mink, chickens and pigs. Three experiments were conducted one with each of the species. Four diets were used in each of the experiments one served as control and in the others increasing contents of either fish meal (mink and chicken) or soybean meal (pig) were replaced with BPM so the level of protein was the same in all diets. During the growth period four (pig) or five (mink and chicken) balance and respiration (indirect calorimetry) experiments were conducted. In the mink and pig experiment the protein turnover was determined by the end-product methods using [^{15}N] glycine as tracer. Furthermore was the content of purine base derivatives analysed in the urine.

The experimental diets were well accepted by the animals except for the mink diet where 60% of the digestible nitrogen derived from BPM. The apparent digestibility of nitrogen decreased significantly in the mink and pig experiments as dietary BPM content increased. In both the mink and pig experiments the retention of nitrogen (RN) was not affected by diet. In the chicken experiment RN was the same on all diets with BPM, which was significantly lower than on the control diet. This was caused by slightly higher nitrogen content in the control diet than the diets with BPM in chicken experiment. The heat production was not affected by increasing content of dietary BPM. The retention of energy was affected only by diet in the mink experiment; mink on the highest inclusion level of BPM had zero retention of energy, while mink on the other diets has positive energy retention. The protein turnover increased significantly with increasing content of dietary BPM in the mink experiment. In the pig experiment no significant differences in protein turnover were observed. With increasing content of BPM in the diet the content of nucleic acid nitrogen also increased. This led to a higher excretion of allantoin in the urine. The mink had higher excretion of purine base derivates in relation to metabolic body size than the pig. The decomposition and excretion patterns of the purine bases differed between the two species.

It was concluded that up to 40% (mink), 20% (chicken) and 50% (pig) of the nitrogen can be derived from BPM without any negative effects on the protein and energy metabolism. Increasing content of BPM in diet led to higher urinary excretion of allantoin in the mink and pigs.

Sammendrag

Bakterielt protein mel (BPM) er et nyt proteinfodermiddel produceret ved fermentation af naturgas som energi og karbon kilde og ammoniak som nitrogen kilde ved brug af *Methylococcus capsulatus* (Bath) (>90%), *Ralstonia* sp., *Brevibacillus agri* og *Aneurinibacillus* sp. Den bakteriel biomasse er steriliseret og sprøjtetørret for at opnå et tørt og stabilt produkt.

Formålet med denne afhandling er at evaluere effekten af stigende mængder af BPM i foderet på protein og energi omsætningen hos mink, kyllinger og grise i vækst. Der blev udført tre forsøg en med hver dyreart. Der blev anvendt fire diæter i hvert af forsøgene, en af diæterne fungerede som kontroldiæt og i de andre diæter blev stigende mængder af enten fiskemel (mink og kylling) eller sojaskrå udskiftet med BPM således at protein niveauet var det samme i alle diæter. I løbet af vækstperioden blev der udført henholdsvis fire (grise) og fem balance og respirationsforsøg. I forsøgene med mink og grise blev proteinomsætningen målt ved hjælp af slutprodukt metoden til dette formål blev [^{15}N]glycine anvendt som tracer. Desuden blev indholdet af purine base derivater målt i urinen.

Dyrene åd forsøgsdiæterne uden problemer undtagen minkfoderet, hvor 60% af det fordøjelige nitrogen kom fra BPM. Den tilsyneladende fordøjelighed af nitrogen faldt signifikant i mink- og griseforsøget, når indholdet af BPM i foderet blev øget. I både mink- og griseforsøget var aflejringen af nitrogen ikke påvirket af diæten. I kyllingeforsøget var aflejring af nitrogen den samme for alle diæter med BPM, hvilket var signifikant lavere end kontroldiæten. Årsagen var at kontroldiæten havde et lidt højere nitrogen indhold end diæter med BPM. Varmeproduktionen var ikke påvirket af stigende indhold af BPM i foderet. Aflejring af energi var kun påvirket i minkforsøget; minkene på det højeste iblandingsniveau af BPM havde en energiaflejring på nul, mens minkene på de andre diæter havde en positiv energiaflejring. Proteinomsætningen steg med stigende indhold af BPM i foderet i mink forsøget. I forsøget med grise blev der ikke observeret nogen forskelle i proteinomsætningen. Når indholdet af BPM øges i foderet stiger mængden af nukleinsyre i foderet også. Dette medførte at udskillelsen af allantoin i urinen steg. Minkene havde en større udskillelse af purine base derivater i forhold til metabolisk kropsstørrelse end grisene. Nedbrydningen af og udskillelsesmønstret af purine baserne var forskellige mellem de to arter.

Det blev konkluderet at op til 40% (mink), 20% (kylling) og 50% (gris) af nitrogenet kan stamme fra BPM uden negative effekter på protein og energiomsætningen. Stigende mængder af BPM i foderet medførte en højere udskillelse af allantoin i urinen hos minkene og grisene.

List of publications included in the thesis

This thesis is based on four papers. The three first papers are about the effect of BPM on the protein and energy metabolism during the growth period in mink, chickens and pigs. The last paper is a comparative study of the protein turnover and nucleic acid metabolism in mink and pigs. The papers would be referred to by their capital letters.

A **Hellwing A.L.F., Tauson A.-H., Ahlstrøm Ø., and Skrede A. 2005.** Nitrogen and energy balance in growing mink (*Mustela vison*) fed different levels of bacterial protein meal produced with natural gas. Archives of animal nutrition 59: 335-352

B **Hellwing A.L.F., Tauson A.-H., and Skrede A. 2005.** Effect of bacterial protein meal on protein and energy metabolism in growing chickens. Poultry Science. Submitted

C **Hellwing A.L.F., Tauson A.-H., Kjos N.P., and Skrede A. 2005.** Bacterial protein meal in diets for growing pigs – effects on protein and energy metabolism. Animal Science. Submitted

D **Hellwing A.L.F., Tauson A.-H., Skrede A., Kjos N.P. and Ahlstrøm Ø. 2005.** Bacterial protein meal in diets for pigs and mink – protein turnover and urinary excretion of purine derivatives. In Manuscript

Introduction

The demand for high quality protein feedstuff both for animals and humans is increasing worldwide because of an increase in the human population, limitation in fish available for production of fish meal, ban of meat-and bone meal and other animal by-products in many countries owing to bovine spongiform encephalitis and gene-modification of soy beans, which especially in the European countries have not been well accepted as animal feed and for human consumption. Therefore, new protein sources based on fermentation of different by-products from the industry may have the potential to enter the market. Although a new protein source not is used to any great extent on farm level the product in itself may be of scientific interest because of the different dietary components in the feedstuff, which may affect the metabolism of the animal.

Before a new protein source is used for farm and companion animals the quality of the product has to be evaluated. Prerequisites for a protein feedstuff to be considered of high nutritional quality include a good palatability, a high biological value, harmlessness and a positive influence on product quality. Performance and digestibility studies give valuable information about expected growth rate, feed conversion rate and digestibility of the different nutrients in the diet. For a new protein source to be considered of high quality it must also be proved to sustain a high rate of nitrogen retention and not cause elevated heat production.

Bacterial protein meal (BPM) with the trade name Bioprotein (Norferm DA, Norway) is a new protein source. BPM is produced by fermentation of natural gas as energy and carbon source and ammonia as nitrogen source by *Methylococcus capsulatus* (Bath) (>90%), *Ralstonia* sp., *Brevibacillus agri* and *Aneurinibacillus sp.* (Skrede et al., 1998). The bacterial biomass is heat sterilised and spray-dried to obtain a dry and stable product. The dry matter content of the final product is about 96% dry matter, 70% crude protein, 10% lipids and 7% ash (Skrede et al., 1998). Previously other types of microbial protein fermented on other substrates have been evaluated; many of them are not produced anymore. For reviews see Snyder (1970), Kihlberg (1972), Schulz and Oslage (1976), Litchfiel (1977), Waldroup (1981), Solomons (1983), Litchfiel (1983), Goldberg (1985), Giec and Skupin (1988), Kuhad et al. (1997), and Anupama and Ravidra (2000).

During the last decade BPM has been investigated in a number of performance studies in different monogastric species and fish, and has been found to be a promising new protein source: A dietary supply of BPM providing up to one third of the N intake sustained production performance and animal health in slaughter chickens (Skrede et al., 2003) and blue foxes (Skrede and Ahlstrøm, 2002). When BPM made up about 50% of dietary N no adverse effects were reported for growing-finishing pigs (Øverland et al., 2001) or Atlantic salmon (Storebakken et al., 2004; Berge et al., 2005), but an inclusion level of 40% - 50% of the dietary N from BPM has resulted in reduced performance during the piglet period in some experiments (Øverland et al., 2001; 2004). The apparent faecal digestibility of nitrogen in BPM has been found to be 79%, 85,4% and 80.5% in mink, pigs and chickens respectively (Skrede et al., 1998). Comparison of diets where 50% of the nitrogen derived from BPM with other protein sources in the blue fox has shown that a diet with BPM had the same apparent total tract digestibility of nitrogen as meat meal and soybean meal but lower than fish meal (Vhile et al., 2005).

Although the performance of animals fed BPM and the digestibility of BPM is identical to other high quality protein sources, it has to be proved that BPM gives the same retention of nitrogen (RN), heat production (HE) and retention of energy (RE) as other high quality protein sources i.e. fish meal and soybean meal. The content of non amino acid nitrogen in BPM is high. About 12% of the nitrogen in BPM derived from RNA and DNA. This level being low compared to that of many other single-cell proteins of bacterial origin (Braude et al., 1977; Tiermeyer et al., 1981; Rumsey et al., 1991; Kiessling and Askbrandt, 1993). Compared with fish meal and soybean meal the level in BPM is much higher (Greife, 1984a; Herbel and Montag, 1987; Devresse 2000). Only animal products like lymph nodes and pancreas have the same content of RNA and DNA on dry matter base as BPM (Herbel and Montag, 1987). These products can be found in slaughter-house offal and meat and bone meal used for instance in mink diets but not as single feedstuffs.

From studies in rats and pigs it is known that the protein turnover may be affected by the quality of the protein (Schadereit et al., 1999; Saggua et al., 2000). Therefore the protein turnover was measured with the end-product methods using [^{15}N]glycine as tracer. This method is easy to apply in difficult situations (Duggleby and Waterlow, 2005).

The dietary RNA and DNA are decomposed to nucleic acid in the lumen of the intestine and the digestibility is probably high (Shannon and McNab 1973; Roth and Kirchgessner, 1978; Greife and Molnar, 1980). The nucleic acids are absorbed as nucleosides or free purine and pyrimidine bases (Wilson and Wilson, 1958, 1962; Privat de Garilhe, 1967; Berlin and Hawkins, 1968; Barnard, 1969). Investigations with different types of bacterial protein, yeast RNA, different nucleotides, nucleosides and purine bases and pyrimidine bases have shown that plasma concentration of allantoin, uric acid and creatinine may be increased (Roth and Kirchgessner, 1977a,b; Yokozawa et al. 1982, 1983; Greife et al. 1984; Brulé et al., 1988), the utilisation of dietary purine and pyrimidine bases differed (Savaiano and Clifford, 1978; Greife and Molnar, 1978a,b; Ho et al. 1979; Greife, 1984b; Greife and Molnar, 1983, 1984a,b; Berthold et al. 1995) and urinary excretion of purine base derivates were increased (Heaf and Davis, 1976; D'Mello et al. 1976; Braude et al. 1977; Roth and Kirchgessner, 1977a,b, 1978; Yokozawa et al. 1982, 1983; Greife et al. 1984; Brulé et al., 1988).

Validation of protein quality and purine base metabolism in more than one species at different times during the growth period makes it possible to draw stronger conclusions of the results. Mink, pigs and chickens are all important production animals in the Nordic countries. The differences in requirements for nutrients, anatomy, growth rate and physiology of the three species might lead to different results.

Objectives of the thesis

- To evaluate effects of bacterial protein meal from natural gas on the protein and energy metabolism in mink, chickens and pigs throughout the growth period when fed iso-nitrogenous and iso-energetic diets.
- To evaluate effects of bacterial protein meal from natural gas on protein turnover in mink and pigs by means of end product method using [^{15}N]glycine as tracer.
- To evaluate effects of bacterial protein meal from natural gas on intake of purine and pyrimidine bases and urinary excretion of purine base derivatives in mink and pigs.

Bacterial protein meal – production and composition

Norferm DA produces BPM by continuous fermentation of natural gas as energy and carbon source and ammonia as nitrogen source by *Methylococcus capsulatus* (Bath), *Ralstonia* sp., *Brevibacillus agri* and *Aneurinibacillus* sp. (Skrede et al, 1998) in a loop fermenter at 45 °C (Norferm, 2005). The liquid from the fermenter containing the biomass is centrifuged, ultra-filtrated, spray-dried and heat treated to obtain a dry and storage stable product.

More than 90% of the biomass from BPM is derived from the gram-negative bacterium *Methyloccoccus capsulatus* (bath). This bacterium has some characteristic traits, which have an impact on the chemical composition but also on the accessibility of the nutrients in the cytoplasm. Foster and Davis (1966) were the first who described the group of bacteria, which *Methyloccoccus capsulatus* (bath) belongs to. *Methyloccoccus capsulatus* (bath) is non-motile, theromtolerant diplococci with a diameter of approximately 1.0-2.0 μm (Foster and Davis, 1966, Smith et al., 1970). Many bacteria store carbon as poly-β-hydroxybutyrate (PHB) if the nitrogen supply is limited but Foster and Davis (1966) could not detect it in methane-utilizing bacteria. In pigs PHB has been shown to have a digestibility of about 30% (Brune and Nieman, 1977a) and in rats the growth rate was depressed and mortality increased, if the PHB content in the diet was more than 30% (Brune and Nieman, 1977b). The polysaccharides in the cell wall, which surrounds *M. capsulatus* (bath) are practically insoluble in water under growth conditions (50 ºC) but can be dissolved at 100 ºC (Foster and Davis, 1966) and therefore it may expected that the polysaccharides in the cell wall of *M. capsulatus* (bath) cannot be dissolved in the intestine, where the temperature is about 37 ºC and this may affect the digestibility. Besides the membranes, which surround the cell, *Methyloccoccus capsulatus* (bath) also has an intracytoplasmic membrane system, which possibly plays a role in transfer of energy (Smith et al., 1970). The amino acid, lipid and carbohydrate composition of the cytoplasm, and different cell wall components, are not known.

BPM is described as light brown/reddish with neutral smell and a particle size of 150-200 μm. Chemical analyses have been performed on BPM (Skrede et al., 1998; Øverland et al., 2001; Storebakken et al, 2004; Schøyen et al., 2005; Norferm 2005; Kjos, unpublished data) and the results are given in Table 1. In the same table the chemical composition of fish meal (Skrede et al 1998) and soybean meal (SBM) are given (Kjos, unpublished data) for comparison.

Protein and amino acids: The crude protein content of BPM is a somewhat lower than in fish meal (Table 1) and higher than in SBM. The processing of the final BPM was changed during the experimental period. The BPM used in the pig experiment (paper C/Kjos, unpublished data) was pelleted and during this process 1% soy oil was added (Øverland et al., 2004). This caused a slightly lower crude protein content (Kjos, unpublished data).

The amino acid profile is similar to that of fish meal, except that the lysine content is somewhat lower and the tryptophan content higher (Skrede et al., 1998). Compared to SBM the amino acid composition of BPM mainly differs regarding the contents of S-containing amino acids with a somewhat higher content of methionine but lower content of cystine, the total S-containing amino acids being slightly higher in BPM. For other essential amino acids only minor differences between SBM and BPM have been found (Table 1).

Fat: About 10% of the dry matter of BPM is fat. Phospholipids make up the main lipid fraction in *Methyloccoccus capsulatus* (bath). Phosphatidylethnolamine, phosphatidylglycerol, phosphatidylcholine and cardiolipin make up 74%, 13%, 8% and 5% of the phospholipids, respectively (Makula, 1978). The two most common fatty acids are 16:0 and 16:1 (Makula, 1978, Jahnke, 1992). Also steroids and squalene have been detected in *Methyloccoccus capsulatus* (bath) (Bird et al., 1971). The content of fat in the batch of BPM used in the pig experiment (paper C) was slightly higher, because soy oil had been added during the pelleting process (Øverland et al, 2004).

Carbohydrate: Nitrogen free extract and fibre make up about 10% of the dry matter (DM) in BPM (Table 1) but no further information is available on the composition. In general the cell wall of different gram-negative bacteria is characterized mainly by different heptoses, glucose, galactose, *N*-acetylglucosamine, rhamnose and mannose as well as some unusual dideoxy sugars (Madigan et al. 2000).

RNA and DNA: About 10% of the DM in BPM is derived from RNA and DNA. The content is low compared to many other single-cell proteins of bacterial origin (Braude *et al.*, 1977; Tiermeyer *et al.*, 1981; Rumsey *et al.*, 1991; Kiessling and Askbrandt, 1993). The content of the different pyrimidine and purine bases in BPM has not been analysed in this experiment but the content in the

diets has been analysed (papers 2, 3 and 4). The mol% G + C of the DNA is 62.5% in *Methyloccoccus capsulatus* (Whittenbury and Krieg, 1984).

Minerals: The ash content of BPM is lower than in fish meal and higher than in SBM. The content of phosphorus is high, because of the high content of RNA and DNA. The phosphorus content is 19.5 g/kg DM (Norferm, 2005). Kjos (unpublished) has determined the content to 10.4 and 16.4 g/kg DM in the two batches of BPM used in the pig experiment (paper C). In SBM the phosphorus content in the batches used in the pig experiment were 6.4 and 5.4 g/kg DM and this is somewhat lower than BPM. In fish meal the phosphorus content is 23.9 g/ kg DM (Andersen and Just, 1990), which is higher than in BPM. The differences between fish meal and BPM regarding phosphorus are that in BPM phosphorus is mainly found in lipids, RNA and DNA whereas in fish meal it is mainly found in hydroxyapatite.

Energy: The gross energy content of BPM is claimed to be 22.1 MJ/kg DM (Norferm, 2005) and in our laboratory the value has been determined to 24.0 MJ/kg DM.

Table 1. Chemical and amino acid composition of bacterial protein meal (BPM), fish meal and soy bean meal (SBM). The chemical composition is given as g/kg and amino acids as g/16 g N.

	BPM						Fish meal	SBM
	Norferm 2005[1]	Skrede et al. 1998[2]	Øverland et al. 2001[2]	Storebakke et al. 2004[2]	Schøyen et al. 2005	Kjos, unpublished[3,4]	Skrede et al. 1998	Kjos, unpublished[3]
Dry Matter	95.0	95.9	95.8	98.6	93.6	93.9	91.6	90.0
Crude Protein	67.1	70.2	69.2	67.1	67.5	65.6	73.8	46.1
Crude Fat	9.3	10.3	10	10.3		11.7	11.0	2.2
Ash	6.7	8.1	8.0	7.9		6.9	12.0	5.7
Crude fibre	0.7					0.6		6.5
N-free extract	11.2					9.3		29.6
RNA		7.3						
DNA		2.2						
Lysine	6.5	6.1	5.8	5.1	5.1	5.3	8.6	6.2
Methionine	2.8	3.0	2.7	2.2	2.4	2.5	3.0	1.3
Cysteine	0.6	0.6	0.6	0.8	0.6	0.6	0.6	1.4
Threonine	4.7	4.8	4.4	4.1	3.8	4.1	3.3	3.8
Tryptophan	2.2	2.1		1.9	1.7	3.0	1.0	1.4
Leucine	7.8	7.8	7.5	7.4	7.1	7.5	8.3	7.8
Isoleucine	4.8	4.8	4.5	4.0	3.7	4.5	5.0	5.0
Valine	6.4	6.1	5.8	5.5	5.7	6.0	5.4	5.2
Tyrosine	3.7	3.8	3.6	3.3	3.4	3.7	3.1	4.2
Phenylalanine	4.7	4.4	4.2	4.4	3.9	4.0	4.2	5.1
Histidine	2.6	2.3	2.3	1.9	2.0	2.3	2.2	2.8
Arginine	6.2	6.4	6.2	5.6	6.0	6.5	6.9	7.6
Alanine	7.4	7.3	6.9	6.5	7.9	7.0	6.2	4.4
Aspartic acid	9.2	9.1	8.5	8.1	8.0	8.3	11.3	11.6
Glutamic acid	10.9	10.9	10.3	9.6	10.0	11.1	11.7	19.7
Glycine	5.1	5.3	4.9	4.6	4.5	5.0	6.9	4.3
Proline	4.5	4.3	4.0	3.6	3.5	3.7	5.0	5.1
Serine	3.8	3.8	3.5	3.5	3.2	3.4	3.9	5.4

[1] Data given by Norferm DA, Tjeldbergodden, N-6699 Kjørsvigbugen, homepage: www.norferm.no.
[2] Experimentally produced batch.
[3] Average of analyses performed on the two batches used in the pig experiment (paper C).
[4] Commercially produced batch, which has been pelleted. During the pelleting process 1% soy oil has been added.

Characteristics of the species used in the experiments

Three different species were used in the experiments: The mink (*Mustela vison*), which belongs to the group of mustelidae, the chicken (*Gallus domesticus*), which belongs to the group of birds and the pig (*Sus scrofa*), which belongs to the group of artiodactyla (Stevens and Hume, 1996). The pig and the chicken are ominvores and ingredients used for diet manufacturing for these species were almost the same. The mink is a carnivore and the diets are mainly based on offal from the fish industries and abattoirs and different dry animal by-products.

When chickens are hatched they can be fed the same type of diets as fully-grown chickens. In comparison, mink and pigs give birth to offspring at an earlier stage of development than the chickens and they have to suckle their young. The length and capacity of their digestive tracts increase during the prenatal development (Stevens and Hume, 1996). Mink kits are born more immature than piglets. At birth the mink kits are blind, nearly hairless, undeveloped thermoregulation and with very limited locomotor abilities (Tauson, 1994). Therefore, BPM in the mink and pig experiment first was introduced after weaning. Mink kits used in the experiment (paper A) were weaned 6 weeks after birth and the piglets were weaned during their 5^{th} week of life (paper C).

The three experiments were terminated at different stages of growth and comparison of data between species has therefore to be done with caution. The last balance period in the mink experiment was conducted in November, where the mink is expected to have reaced mature size (Hansen et al., 1991). In the chicken experiment the last period was conducted when the animals where 30 to 34 days old and weighed about 2.1 kg. The highest growth rate of Ross chickens is obtained around day 40 after hatching (Ross, 2002) and this is in agreement with Chwalibog et al. (1985), who determined the age of maximum nitrogen retention to 45 days after hatching. The last period in the pig experiment was conducted when the pigs weighed around 80 kg and this was lower than the normal weight of mature body size. Whittemore et al. (1988) have shown that the highest retention of nitrogen was reached for pigs with a body weight of about 75 kg. Furthermore Chawlibog et al. (1996) have shown the highest retention of nitrogen to occur at 98 kg for pigs of both sexes and Tauson et al. (1998) to be about 135 kg for intact boars.

Furthermore, the genetic selection criteria for of the animals are different: The chicken is mainly selected for a fast growth rate and high feed utilisation (Christensen, 1999). Pigs are also selected for this purpose but also a high meat content and number of pigs per litter are parts of the selection program for Danish pigs (Lauritsen, 2005). The mink is mainly selected for body size but not feed utilisation because a bigger mink has a bigger skin (Christensen, 1999).

Anatomic and physiological differences

From Figures 1a, 1b and 1c it is clear that the anatomy of the three animals also differ. The mink has a very short gastrointestinal tract and the length about 4 times the length of the animal (Tauson, 1988). In pigs the gastrointestinal tract is 14 times the body length (Argenzio, 1995). In the chicken the length of the gastrointestinal tract of 3 kg broiler is 217 cm (Denbow, 2000), if the chicken is about 46 cm in length (Stevens and Hume, 1996), the gastrointestinal tract is about 5 times longer than the animal, which is less than in the pig and nearly the same as the mink. However, the chicken gastrointestinal tract differs from that of the mink. Chickens have two ceacas with a length of about 20 cm, whereas the mink has no cecum at all. In the cat, which has a gastrointestinal tract which is very similar to the mink, the small intestine makes up 83% of the total length of the intestine, in pigs it is 78% (Argenzio, 1995) and in chickens 93% (Denbow, 2000).

The role of the colon in the chicken differs from both the mink and pig (Denbow, 2000). The chicken has not separate excretion of faeces and urine as the mink and pig but it excrets both residues from digestion of feed and urine as droppings. The ceacas are important in for digestibility of nutrients chicken. Urine from the cloaca can be carried into the ceacas where it can be degraded by micro organism (Denbow, 2000). In the pig colon undigested material can be degraded by micro organisms. In the mink the content of microbes in the intestine is much lower than in the pig (Williams et al., 1998).

The metabolism of nutrients and importance of the different nutrients in the energy metabolism also differed between the animals. There are however, two things, which are of importance in connection with our experiments. The first is the excretion of nitrogen. In mink and pigs nitrogen is excreted as urea and ammonium and purine bases are decomposed to allantoin (Figure 2). In the chicken nitrogen is mainly excreted as uric acid and purine bases are also decomposed to uric acid.

The α-amylase activity in the mink intestine is low (Elnif et al., 1988) and also microbial activity is low (Williams et al., 1998). Mink can therefore only digested simple carbohydrates and to ensure the glucose homoeostasis the supply of amino acids has to be high. The oxidation of protein is therefore often higher in mink than in pigs (Chwalibog et al., 1998).

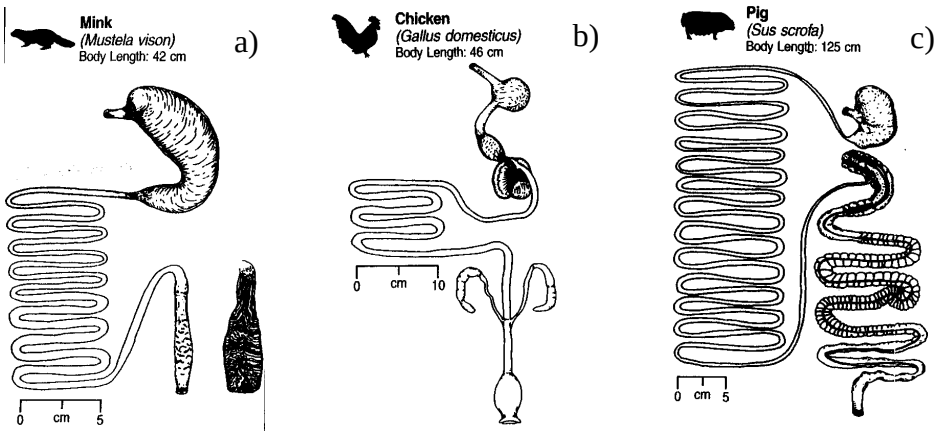

Figure 1. Gastrointestinal tract of the mink (a), chicken (b) and the pig (c) from Stevens and Hume (1996).

Material and methods

The experimental designs, diets and methods are described in details in papers A, B, C, D. However, some further diet information on diet composition is given in Table 2. In the general discussion the diets are numbered differently from the papers and in Table 2 both the numbers used in the general discussion and the numbers used in the respective papers are given. In general, the energy metabolism has been calculated in kJ except for all values in paper B being presented in kcal because of journal standards. All values regarding energy metabolism in the general discussion are given in kJ.

Table 2. Content of bacterial protein meal (BPM) and nitrogen as is and on dry matter (DM) basis, % nitrogen (N) derived from BPM and purine and pyrimidine bases and diet codes used in the general discussion and in the papers.

Diet codes used in the general discussion	M1	M2	M3	M4	C1	C2	C3	C4	P1[1]	P2[1]	P3[1]	P4[1]
Composition and chemical content of the diets												
BPM content as is	0	4.5	9.0	13.5	0.0	2.0	4.0	6.0	0	5.2	10.1	15.3
DM	39.4	39.4	39.6	40.2	90.3	90.3	90.4	90.6	91.9	91.7	91.8	91.6
Nitrogen as is	2.5	2.4	2.4	2.4	3.7	3.5	3.4	3.4	3.2	3.2	3.2	3.3
BPM on DM basis	0.0	11.4	22.7	33.6	0.0	2.2	4.4	6.6	0.0	5.7	11.2	16.9
Nitrogen on DM basis	6.3	6.1	6.1	6.0	4.1	3.9	3.8	3.8	3.5	3.5	3.5	3.6
% of N derived from BPM	0	20	40	60	0	7	13	20	0	17	35	52
% of N derived purine and pyrimidine bases	5.8	7.7	9.9	11.2	2.9	3.6	4.1	4.9	2.7	5.6	7.7	9.7
Diet codes used in												
Paper A	Diet I	Diet II	Diet III	Diet IV								
Paper B					D0	D2	D4	D6				
Paper C									BP0	BP5	BP10	BP15
Paper D	M1	M2	M3	M4					P1	P2	P3	P4

[1] In the pig experiment both starter and growing-finishing diets were used (paper C). The protein level was slightly lower in the growing-finishing diet than in the starter diet. The values given in the table are the highest value of the two.

Summary of papers presented

A. Nitrogen and energy balance in growing mink (*Mustela vison*) fed different levels of bacterial protein meal produced with natural gas

The aim of the experiment was to evaluate how increasing dietary content of BPM affected nitrogen and energy metabolism and water turnover.

Sixteen male mink kits of the standard brown colour type were randomly fed one of the four experimental diets. One diet served as control diet (diet I) and high-quality fish meal was replaced with increasing levels of BPM on basis of digestible nitrogen so 20% (Diet II), 40% (Diet III) and 60% (Diet IV) of the digestible nitrogen derived from BPM. These diets were fed during the five balance periods as close as possible to ad libitum feeding. Nitrogen balance, water balance and respiration experiments (indirect calorimetry) were conducted when the mink kits were in their respective 10^{th} (period 1), 15^{th} (period 2), 18^{th} (period 3), 24^{th} (period 4) and 29^{th} (period 5) week of life.

The feed intake on Diet IV was significantly lower than on the other diets. The intake of nitrogen on Diet IV was also lower but the differences were not significant (P=0.07). The water balance was the same on all diets but intake of drinking water and faecal water increased whereas dietary water and urinary water decreased with increasing BPM content in the diet. The apparent digestibility of nitrogen, fat, carbohydrates and energy decreased significantly with increasing dietary content of BPM. The retained nitrogen was 0.45, 0.54, 0.52 and 0.40 g/kg$^{0.75}$ on Diets I, II, III and IV, respectively, the observed differences between diets being non-significant (P = 0.06). The amount of metabolisable energy available for production was significantly reduced on diet IV (P = 0.001). Although mink kits on diet IV have a lower feed intake the heat production (HE) was the same on all diets (P = 0.78). HE was lowest on diet II with 645 kJ/kg$^{0.75}$ and highest on diet I with 665 kJ/kg$^{0.75}$. Retained energy was approximately 150–160 kJ/kg$^{0.75}$ on Diets I to III, whereas it was –11 kJ/kg$^{0.75}$ on Diet IV, the differences being significant (P < 0.001). The amount of HE from oxidation of protein decreased from 32.7% on Diet I to 26.6% on Diet IV, and oxidation of fat increased from 53.8% on Diet I to 63.5% Diet IV. It was concluded that up to 40% of the digestible protein could be derived from BPM.

B. Effect of bacterial protein meal on protein and energy metabolism in growing chickens

The aim of this study was to evaluate whether increasing dietary content of BPM in the diet affected the nitrogen and energy metabolism and the carcass composition of growing slaughter chickens.

Seventy-two Ross male chickens were randomly allocated to four experimental diets, each in three replicates. One of the diets served as control diet with no BPM (D0). In the other diets fish meal was replaced by BPM on basis of nitrogen content and about 6.5% (D2), 13.2% (D4), and 19.9% (D6) of the nitrogen was derived from BPM. Five balance periods were conducted when the chickens were 3–7, 10–14, 17–21, 23–27, and 30–34 days old. During the same periods, 22-hour respiration experiments (indirect calorimetry) were performed with groups of 6 chickens (period 1), 5 chickens (period 2), and 1 chicken (periods 3–5). After each balance period, one chicken in each cage was killed and the carcass weight was recorded. Chemical analyses were performed on the carcasses from periods 1, 3, and 5.

Weight gain, feed intake, and feed conversion rate were found to be similar for all diets. Intake of nitrogen was the same on the diets with BPM, which were significantly lower than the control diet ($P = 0.01$). This was caused by somewhat higher nitrogen content in the control diet. The higher intake of nitrogen also led to higher nitrogen retention on D0 (1.59 g N/kg$^{0.75}$/day). The nitrogen retention on D2, D4, and D6 were 1.44 g, 1.52 g, and 1.50 g N/kg$^{0.75}$/day, respectively and did not differ significantly. The amount of metabolisable energy available for production was the same on all diets ($P = 0.39$). The heat production was not affected by diet ($P = 0.92$) and was between 189 kcal (792 kJ)/kg$^{0.75}$/day (D6) to 210 kcal (877 kJ)/kg$^{0.75}$/day (D4). The energy retention was also the same on all diets ($P=0.88$). The respiratory quotient was between 0.92 and 0.94 and was not affected by diet ($P = 0.90$). Oxidation of fat and carbohydrates was the same on all diets. The dry matter, nitrogen, fat and energy content of the carcasses were the same for all diets and this was in line with the findings for protein and energy metabolism of the study. It was concluded that up 20% of the nitrogen could be derived from BPM without negative effects on the overall nitrogen and energy metabolism in chickens.

C. Bacterial protein meal in diets for growing pigs – effects on protein and energy metabolism

The aim of this study was to investigate the effect of increasing dietary content of BPM on the protein and energy metabolism in pigs from weaning until a live weight of 80 kg.

Four litters with four castrated male pigs were bought when the pigs weighed about 8 kg. The litters were divided into two blocks according to time of weaning. A pig from each litter was fed one of the four experimental diets. Soybean meal was replaced with BPM on basis of digestible protein, and the BPM contents were 0%(BP0), 5%(BP5), 10%(BP10) and 15%(BP15) in the four diets, corresponding up to 0%, 17%, 35% and 52% of the digestible nitrogen derived from BPM, respectively. Four balance periods were conducted when the pigs weighed 9.5 kg, 20.7 kg, 45.3 kg and 77.2 kg at the start of the respective balance periods. During the same periods 22-hours respiration experiments (indirect calorimetry) were performed.

Weight gain, feed intake and feed conversion rate as well as the intakes of nitrogen and energy were the same for all diets. The apparent digestibility of nitrogen was significant lower on BP10 than on BP0 whereas the apparent digestibility of energy and carbohydrates were similarly on all diets. Apparent digestibility of fat increased significantly with increasing BPM. The amount of digestible nitrogen was significantly lower on BP10 than on BP5 but this did not affect the retention of nitrogen, which was 1.50, 1.53, 1.33 and 1.46 g $N/kg^{0.75}$ on BP0, BP5, BP10 and BP15, respectively. The utilisation of digestible nitrogen for retention was lowest on BP10 but did not differ significantly from the other diets ($P = 0.15$). Neither metabolisable energy ($P = 0.22$) nor heat production ($P = 0.29$) were affected by diet. Retention of energy was 620, 696, 613 and 664 $kJ/kg^{0.75}$, differences being non-significant ($P = 0.25$). N-free respiratory quotient was between 1.04 on BP10 and 1.07 on BP0, which indicates that the pigs have retained fat. About 15% of the heat production came for protein oxidation, about 4% from fat oxidation and the rest from oxidation of carbohydrates, differences between diets being non-significant. It was concluded that the overall protein and energy metabolism in growing pigs were not affected when up to 50% of the dietary N was derived from BPM.

D. Bacterial protein meal in diets for pigs and mink – protein turnover and urinary excretion of purine base derivatives

The aim of this study was to see how increasing levels of dietary BPM to mink and pigs affected the protein turnover and nucleic acid and creatinine metabolism.

In each experiment sixteen animals were allocated to four experimental diets. One of the diets served as control diet without BPM (Mink(M)1, Pig(P)1) and then increasing levels of BPM replaced fish meal (mink) or soybean meal (pig) so that up to 17% (P2), 20% (M2), 35%(P3), 40% (M3), 52% (P4), and 60% (M4) of the digestible N was derived from BPM. In the mink experiment the five balance periods were conducted when the animals where in their respective 10^{th} (period 1), 15^{th} (period 2), 18^{th} (period 3), 24^{th} (period 4) and 29^{th} (period 5) week of life. In the pig experiment four balance periods were conducted when the animals weighed about 10.1 ± 1.8 kg (period 1), 21.9 ± 4.0 kg (period 2), 47.6 ± 4.7 kg (period 3) and 79.3 ± 5.0 kg (period 4). The content of creatinine and purine base derivatives were determined in the collected urine. The protein turnover was measured in all balance periods in the mink experiment. In the pig experiment it was only measured in periods 2 and 4. The protein turnover was measured by means of the end-product methods using [^{15}N]glycine as tracer and urinary nitrogen as end-product.

In mink the protein flux, synthesis and breakdown increased significantly with increasing dietary level of BPM. In the pig no diet effects were observed on the protein turnover. The net protein synthesis in the mink (P = 0.08) and pig experiments (P = 0.65) was not affected by diet. The intake of nucleic acid nitrogen (NAN) increased from 0.15 g/kg$^{0.75}$ on M1 to 0.26 g/kg$^{0.75}$ on M3 and M4 in the mink experiment and from 0.08 g/kg$^{0.75}$ on P1 to 0.33 g/kg$^{0.75}$ on P4 in the pig experiment. The increased intake of NAN led, in both experiments, to an increased excretion of allantoin. Analysis of the species effects showed that the mink excreted 1.72 g/kg$^{0.75}$ of allantoin, which was significantly more than the pig, which excreted 0.95 g/kg$^{0.75}$ of allantoin. In mink about 96% of the excreted purine base derivates was allantoin whereas it in pigs was 93%. The mink excreted more purine base derivatives than ingested purine bases whereas the pigs on M2, M3 and M4 excreted less than ingested. It was concluded that increasing dietary content of BPM increased the protein turnover in mink but not in pigs. The excretion of allantoin increased with increasing dietary content of BPM

General discussion

During the three experiments measurements were carried out on the same animals four to five times. In general, the period effects can be explained by the physiological development of the animal. Only in few cases an interaction effect between diet and period were found. These effects could not be related directly to the BPM in the diet.

Performance

In the mink experiment the diets were only fed during the balance periods, which consisted of an adaptation period and a balance period (paper A). As the experimental diets only were fed during short periods it is not possible to conclude anything about the performance of the animals. Ahlstrøm et al. (2002) observed a decreased performance of mink kits during the first weeks after weaning when they were fed a diet with 8% BPM, corresponding to 42% of the nitrogen was derived from BPM.

In the chicken and pig experiments the animals were fed the experimental diets from the start to the end of each experiment (papers B and C). No differences were observed in growth rate, feed intake and feed utilisation. Skrede et al. (2003) have observed the same performance in chickens as long as the diet contained less than 6% BPM, corresponding to 20% of the nitrogen derived from BPM. Øverland et al (2004) have used the same diets as used in our pig experiment and have observed a somewhat lower growth rate of pigs fed P4 in the piglet period. However, body weight gain and feed intake were also registered between balance periods in the pig experiment (unpublished data) and our data could not confirm the findings by Øverland et al. (2004).

Intake of feed and nutrients

The feed intake on the different experimental diets was similar within species except on M4 in the mink experiment (paper A). The lower feed intake on M4 was observed in all five balance periods conducted. In the first period the feed intake on M4 was only 0.56 of the intake on M1. However, the weight of the animals on M4 was also significantly lower than on M1 and M2. If this was taken into account and the feed intake was calculated on basis of metabolic body size the differences were

less pronounced, but still in period 1, 4 and 5 the intake on M4 was only 0.75-0.8 of the intake on the control diet (M1). It is not known why the feed intake on M4 was depressed. However, a depression in feed intake has also been observed on diets with methanol-grown bacterial protein in mink (Helgebostad, 1976; Skrede, 1976) and chickens (D'Mello and Acamovic, 1976; Plavnik et al., 1981). It was observed that the mink diets got stickier with increasing dietary content of BPM, findings concurring with Helgebostad (1976) for a diet based on methanol-grown bacterial protein. D'Mello and Acamovic, (1976) and Plavnik et al., (1981) related this stickiness to the adhesive properties of the protein, when it was moistened. In the pig experiment the pelleted diets were suspended in water (paper C) and no differences in consistency were observed between diets. The differences in consistency with increasing dietary BPM content observed in the mink experiment but not in the pig experiment may be an effect of the manufacturing process and the ingredients in the diets. The pig diets were manufactured as dry diets and then suspended in water whereas the mink diets were manufactured as wet diets. The BPM content on M4 in the mink experiment was 34% on DM basis, which was much higher than the 17% on P4 in the pig experiment (Table 2). Also ingredients used for diet composition differed, in the pig diets mainly vegetable ingredients were used whereas in the mink diets mainly ingredients of animal origin were used.

The intake of nitrogen (IN) on basis of metabolic body size was the same on all diets in the mink experiment (paper A: Table IV) and also in the pig experiment (paper C: Table 4). In the chicken experiment higher N content on C1 compared with the other diets affected IN (Table 2). The IN was, however, the same on all diets with BPM (paper B: Table 4).

The content of essential amino acids in the diets in both the chicken and pig experiment did not fulfil the requirements given by NRC (1994 (chicken), 1998 (pig)). This has caused a weak and non-significant depression in the RN and utilisation of both IN in the first balance period in the chicken experiment (paper B) and digested nitrogen (DN) for RN in the first and second balance periods in the pig experiment (paper C). In the chicken experiment the biggest differences compared to requirements given by NRC (1994) and Ross (2000) were on C3 and C4. In the pig experiment the lysine level decreased with increasing dietary content of BPM (paper C: Table 2) but the ideal amino acid pattern was fulfilled for all diets (NRC, 1998).

Water metabolism

The quantitative water metabolism was only measured in the mink experiment. Neither the water balance nor the total intake and the total excretion of water were affected by diet (paper A, Figure 1). If the intake was divided into intake of dietary water and drinking water diet M4 differed significantly from the other diets. On this diet the intake of dietary water was significantly lower than the other diets and the intake of drinking water was significantly higher on M4 than on M2 and M3. Also the distribution of the excreted water between urine and faeces was influenced by BPM: the excretion of faecal water increased and urinary water decreased with increasing dietary content of BPM. In chickens the litter quality is of great importance for the health and slaughter quality of the animals. In this study, analyses of the water content in droppings did not differ and neither did the quantity of droppings (unpublished data). This concurred with the results by skrede et al (2003), which either have observed the same or better litter quality in chickens fed diets with BPM. In the pig experiment the total amount of urine and faeces excreted and water content in faeces were the same regardless of dietary BPM content. From the water excretion data from the chicken and pig experiments, it may be concluded that BPM only caused differences in the water turnover in the mink experiment. The changes in water turnover in the mink were probably caused by the lower apparent digestibility of nutrients, which probably have led to a higher water binding capacity of the intestinal content. The higher water intake on M4 was therefore an effect of the higher faecal water excretion. In mink kits Neil (1986) has shown that water absorbents increased faecal water excretion as well as the intake of drinking water. Material not degraded by endogenous enzymes can be degraded by microbes in the colon of the pig and the caeca of the chicken and it is probably therefore the water excretion through faeces and droppings not was affected in these two species. The mink has a colon (Figure 1) but the colon is short and the content of microbes is considerably lower than i.e. the pig and chicken (Williams et al., 1998) and therefore the digestibility of i.e. fibres is low (Børsting et al., 1995; Ahlstrøm and Skrede, 1998; Skrede et al., 2001).

Digestibility

The apparent digestibility of nitrogen (ADN) in the mink and pig experiments was negatively affected by dietary BPM. The effect was most pronounced in the mink where the ADN decreased from 83.3% on M1 to 77.2% on M4. In the pig the faecal ADN only decreased from 78.2% on P1 to

75.8% on P4 but the differences were significant. This concurred with findings in the blue fox by Vhile et al. (2005), who have shown that there was a decrease in ADN of a diet with BPM compared with fish meal but not SBM or meat meal. The ADN of BPM in the mink experiment was by linear regression determined to 64.5% in period 1 and between 73.6% and 75.8% in periods 2 to 5 (paper A, Figure 2), which is lower than the 79% found by Skrede et al. (1998) and 82.3% found by Schøyen et al. (2005). The ADN of high-quality fish meal is between 82-87% (Ahlstrøm et al., 2004) and therefore the observed decrease in ADN with increasing BPM content in the mink experiment may be expected. The ileal ADN of BPM has not been determined in the pig experiment but Skrede et al. (1998) has determined it to 78.1%. This is slightly lower than the ileal ADN of SBM, which is between 79-80% (Pedersen and Boisen, 2002). The slightly lower decrease of ADN in the pig experiment than in the mink experiment is therefore in agreement with the slightly lower differences between ADN of BPM and SBM than between BPM and fish meal.

The lower ADN of BPM compared with both fish meal and SBM is probably related to the cell walls and membranes of *M. capsulatus* (Bath). Compared with other bacteria *M. capsulatus* (Bath) have internal membranes, which play a roll in the energy transfer in the cell (Davies and Whittenbury, 1970; Smith et al., 1970). The cell walls and internal membranes have probably a lower ADN than the cytoplasm. Schøyen et al. (2005) have investigated the effect on ADN in mink fed diets with either autolysed or hydrolysed BPM separated into two fractions depending on the molecular size. The low molecular fraction of both autolysed and hydrolysed BPM had a higher ADN and apparent amino acid digestibility than the high molecular fraction. The high molecular fraction was probably also the fraction, which had the highest content of residues from the cell walls and internal membranes. Digestibility studies in mink fed *M. capsulatus* (Bath) grown on methanol have shown a somewhat higher ADN than BPM and this is probably because of a lower content of internal membranes in the methanol grown *M. capsulatus* (Bath) (Skede, unpublished data). Autolysis of BPM could not alleviate the depression effect of membranes in *M. capsulatus* (Bath) (Schøyen et al., 2005).

The apparent fat digestibility (ADF) differed between the mink (paper A: Table 3) and pigs (paper C: Table 3). In the mink the digestibility decreased and in the pig it increased with increasing dietary content of BPM. The fat content of the pig diet was rather low and increased slightly with increasing content of dietary BPM and therefore endogenous loss may have affected the ADF

(Jørgensen et al., 1992; 1993). If the endogenous loss of fat was 4.4 g/kg DM (Jørgensen et al., 1993) the calculated true digestibility of fat still increased significantly with increasing dietary content of BPM (paper C). In the blue fox the ADF was the same in diets with and without BPM (Vhile et al., 2005; Skrede and Ahlstrøm, 2002). Therefore the decrease in ADF in the mink experiment could not be explained by the current knowledge of BPM.

The apparent digestibility of carbohydrates (ADCHO) decreased significantly with increasing dietary BPM in the mink but did not differ between diets in the pig. Skrede and Ahlstrøm (2002) could not show any differences in ADCHO in the blue fox whereas Vhile et al. (2005) have shown a slightly higher ADCHO in the BPM containing diet compared with the diet with SBM. BPM contains about 12% nitrogen free extract and fibre (Table 1). The carbohydrate content derived from BPM differed considerably in the mink and pig diets. On M4 in the mink experiment 10.5% of the carbohydrates were derived from BPM whereas the content on P4 in the pig experiment was about 3.2%. This and the shorter colon, rapid transit time (Hansen, 1978; Charlet-Lery et al., 1981), lower content of microbes in the intestine (Williams et al., 1998) and the lower digestibility of fibres (Børsting et al., 1995; Ahlstrøm and Skrede, 1998; Skrede et al., 2001) may be the explanation for the lower ADCHO in the mink experiment. Furthermore, it may be expected that the cell wall carbohydrates have a low solubility in the intestine (Foster and Davis, 1966).

It may be concluded that nutrients digestibility in the mink is more depressed than the pig with increasing dietary BPM content. It was probably because the control protein differed between the pig and mink experiment but also the shorter intestine in mink (Figure 1), the rapid transit time in the mink (Hansen, 1978, Charlet-Lery et al., 1981) and the lower content of microbes in the mink intestine than the pig intestine (Williams et al., 1998), which have caused the more pronounced depression in the mink. The digestibility of nutrients in the chicken was not investigated in the chicken experiment but the ADN would probably have been nearly the same on C4 and C1, because ADN of BPM fed to chickens is slightly higher than ADN of BPM fed to the mink (Skrede et al., 1998) and the dietary content of BPM in the chicken experiment was considerably lower compared with the diets in the mink experiment (Table 2).

Nitrogen metabolism

Both on M4 in the mink experiment (paper A:Table IV) and on P3 in the pig experiment (paper C: Table 4) the lower ADN together with a somewhat lower IN led to a lower level of DN. The diets with the lowest DN also had the lowest retention of N (RN). The RN in the mink experiment was between 0.40 g/kg$^{0.75}$ (M4) and 0.54 g/kg$^{0.75}$ (M2). In the pig experiment RN was between 1.33 g/kg$^{0.75}$ (P3) and 1.53 g/kg$^{0.75}$ (P2). In both experiments the differences were non-significant and the P-value was 0.08 in both experiments. In the chicken experiment RN was between 2.58 g/kg$^{0.75}$ (C3) and 2.75 g/kg$^{0.75}$ (C1) and the differences were significant. The higher RN on C1 was probably caused by higher nitrogen content on C1. The diets with BPM did not differ from each other and it may be concluded that BPM did not lead to any changes in RN. In all three experiments increasing dietary content of BPM did not lead to a linear changes in RN. The data also indicate that the capacity for protein synthesis in the three species differed considerably on basis of metabolic body size.

From our data it was not possible to evaluate whether or not the animals have utilised nucleic acid nitrogen (NAN) for nitrogen retention. All diets met or exceeded the minimum protein content for the species (Hansen et al., 1991; NRC 1994; 1998). The content of NAN in the diets with the highest inclusion level of BPM were about 11% (Mink), 5% (Chicken) and 10% (Pig). Animals can synthesize non-essential amino acids from non-protein nitrogen (Eggum and Christensen, 1973). Studies with chickens have shown that purine and pyrimidine bases, within certain limits, can be used as a non-specific source of N for synthesis of non-essential amino acids. Provided the supply of essential amino acids was covered (D'mello, 1979 and 1986). The same has been shown in pigs fed yeast RNA (Roth and Kirchgesser, 1977a and 1978).

The RN differed among species; chickens had the highest RN, pigs were intermediate and mink had the lowest RN on basis of metabolic body size. Chickens also had the highest IN and mink the lowest. The utilisation of IN or DN for RN also differed between species. Chickens had a utilisation of IN for RN between 62.6% and 64.3% (paper B, Table 4), pigs have a utilisation of DN for RN between 54.4% and 59.8% (paper C, Table 4) and the utilisation of DN for RN in the mink was about 20%. The chickens not only had the highest retention but also the highest utilisation. In comparison with the pig and mink the chicken only got 5% of the nitrogen from NAN on C4 and it

can be speculated whether a higher dietary content of BPM would have led to a decreased RN because the pool of digested amino acids then would have decreased.

Both in the mink and the chicken there were interactions between diet and period for RN (papers A and B). In the mink RN on M4 in the first period was about 50% of the other diets, which was related to the lower feed intake observed in this period (paper A). In chickens the RN on C4 was also lowest in the first period but here it was supposed that it was a under supply of essential amino acids than required rather than BPM that has led to this decrease (paper B).

Protein turnover

The protein turnover was measured by means of the end-product methods using [^{15}N]glycine as tracer in the mink and pig experiment (paper D). The species differences in supply of IN were also reflected in the turnover. The synthesis in the mink experiment increased significantly from 15.0 g protein/kg$^{0.75}$on M1 to 20.3 g protein/kg$^{0.75}$ on M4, and in the pig experiment the synthesis was between 30.8 g protein/kg$^{0.75}$ (P4) and 34.0 g protein/kg$^{0.75}$ (P1) but in contrast to the mink the differences were non-significant (paper D: Tables 3 and 4).

The species differences in protein turnover with increasing dietary content of BPM can probably be related to the amino acid composition. The pig diets were balanced regarding the amino acid composition. In the mink diets no attempts were made to balance the amino acid content, but the supply of all essential amino acids may be expected to meet, or exceed the requirements of the mink as the protein level was higher than recommended (Hansen et al., 1991). From studies in pigs it is known that diets with same protein level but increasing content of essential amino acids increased the protein synthesis and breakdown (Gotterbarm et al., 1998). If the amino acid composition of BPM and fish meal as given by Skrede et al. (1998) is compared, the supply of methionine, cystine, tryptophan and threonine increased with BPM content in the diet.

The protein synthesis in the mink experiment was higher than the protein synthesis determined for adult cats fed a high protein diet (Russell et al., 2003). However, there were some differences in the experiments, which may explain the differences observed. First of all the protein turnover may differ between the mink and the cat. The protein turnover for last balance period in the mink

experiment was still higher than the protein turnover for the adult cats, measured by Russell et al. (2003), although it is expected that the mink have reached their mature body size at this point. The protein synthesis may be overestimated in our experiment because 24 hours was used as cut-off time for excretion of label. Furthermore, Russell et al. (2003) calculated their synthesis on basis of excreted label in urea and ammonium whereas we used total N.

The measured protein flux, synthesis and breakdown in the pig experiment was slightly higher than the measurements made by Gotterbarm et al., (1998), Roth et al. (1999), Windisch et al. (2000) and Saggau et al. (2000). The higher protein synthesis can be an effect of higher protein intake in this experiment compared with Gotterbarm et al., (1998), Roth et al. (1999), Windisch et al. (2000) and Saggau et al. (2000), because an increase in protein intake also increase the protein turnover (Reeds et al. 1980, 1981; Fuller et al. 1987).

Nucleic acid metabolism

In the chicken (paper B: Table 2) and the pig (paper C: Table 2) experiments the intake of NAN increased with increasing BPM content of the diet. The same would have occurred in the mink experiment, if the feed intake on M4 has been the same as on the other diets (paper D: Tables 1 and 5). The differences in supply of NAN between the diets with the highest level of BPM and the control diet were greatest in the pig experiment, followed by the mink and then the chicken.

Inntake of both purine bases and the three pyrimidine bases increased significantly in the pig experiment (paper D: Table 6). In the chicken guanine intake was nearly the same on all diets and the thymine intake was highest on C3 but did not differ significantly from C4 (paper B: Table 3). In the mink experiment intake of guanine was significantly lower on M4 compared with M3 and the intake of thymine decreased with increasing dietary content of BPM (paper D: Table 5). The content of the different purine and pyrimidine bases may depend on the feedstuff (Clifford and Story, 1976; Tiemeyer et al., 1981; Herbel and Montag, 1987; Lassek and Montag, 1990). In the mink experiment the content of thymine in fish meal may have been considerably higher than in BPM.

The digestibility of RNA and DNA in pigs and chickens has been shown to be high (Greife and Molnar, 1980, Roth and Kirchgessner, 1978, Shannon and McNab 1973). Barnard (1969) has in the cat, which also is a strict carnivore, shown that RNA is well digested although the content of pancreatic ribonuclease was considerably lower than in the pig and chicken. The digestibility in the mink is probably also high and this is supported by the fact that the excretion of purine derivatives in the mink increased with increasing dietary content of BPM. The digested RNA and DNA are mainly absorbed as different nucleosides and free purine and pyrimidine bases, and already in the enterocytes the catabolism of the nucleosides, purine and pyrimidine bases towards their end products begins (Figure 2 and 3) (Wilson and Wilson, 1958, 1962).

The purine bases are decomposed to allantoin in the mink and the pig whereas in the chicken it is only decomposed to uric acid (Figure 2). However, small amounts of uric acid, xanthine and hypoxanthine were also found in the urine (paper D: Tables 5 and 6). The pyrimidine bases can be decomposed to metabolites which can enter the citric acid cycle or fat metabolism (Michal, 1999), except for cytosine, which either is excreted into urine or degraded by microbes in the intestine (Heaf and Davis, 1976; Kozak et al., 1980).

From the purine base derivatives excretion data and intake of purine bases it is possible to calculate whether or not the animal has excreted more purine derivatives than ingested. On all mink diets and on the pig control diet more purine base derivatives than ingested were excreted. On P2, P3 and P4 in the pig experiment less than ingested were excreted. The total excretion of purine base derivatives was 1.8 $g/kg^{0.75}$ in mink and 1.0 $g/kg^{0.75}$ in the pig although they had nearly the same intake (paper D: Table 7). The purine bases not found in urine in pig can probably have been retained in the pig body. Studies on the purine metabolism in the pig have shown that up to 40% of (8-C^{14})-adenosinemonophosphate (AMP) and 15% of the (8-C^{14})-guanosinemonophosphate (GMP) was retained in the body (Greife and Molar, 1984a). However, Greife and Molar (1984a) have also found label in breath and gastrointestinal tract content. Label in breath was derived from AMP and GMP, which have been microbially decomposed to CO_2 and NH_3. Label in the gastrointestinal content derived either from unabsorbed GMP and AMP or metabolites, which have been excreted into the gastrointestinal tract (Sørensen, 1960, Berlin and Hawkins, 1968). It can be concluded that the pigs probably have retained some of the dietary purine bases, but that excretion of purine derivatives into the gastrointestinal tract may also act as an important excretion route (Greife and

Molnar, 1984a). No similar studies of that kind have been conducted in the mink, but in rats and chickens the retention of labelled (8-C^{14})-AMP and (8-C^{14})-GMP have been lower than the retention in the pig (Greife and Molar, 1983, 1984b). It is clear from our studies of the purine base metabolism in the mink and pigs that retention of dietary purine bases, and the importance of the different excretion routes differed between species.

In mink (paper D; table 5) and pig (paper D, table 6) the allantoin excretion increased with increasing intake of NAN. Allantoin made up 96% of the purine base derivatives excreted in urine in the mink whereas it was only 93% in the pig. The rest was uric acid, xanthine and hypoxanthine. The data suggested that the mink had a more complete metabolism of the purine bases than the pig.

Creatinine

The pattern of creatinine excretion differed between the mink and pig. In the mink the creatinine excretion decreased with increasing dietary content of BPM, but in the pig no differences were seen (paper D). The creatinine excretion may give some information about renal function. Intake of free adenine is known to depress feed intake (rats: Clifford and Story, 1976; Yokozawa et al., 1982 and 1983; Brulé et al., 1988; chickens: Baker and Molitoris, 1974; D'Mello, 1986), increase urine output and decrease creatinine concentration in urine (Clifford and Story, 1976; Savaiano and Clifford, 1978; Yokozawa et al., 1982 and 1983; Brulé et al., 1988). Neither in the mink nor in the pig an increase in urine volume was recorded. It can be concluded that the level of free adenine never exceeded the level where it has a negative effect (Clifford and Story, 1976).

Energy metabolism

The gross energy intake was not affected by diet in the three experiments (paper A: Table 5, paper B: Table 4; paper C: Table V). Metabolisable energy (ME) was in the mink experiment between 652 (M4) and 816 kJ/$kg^{0.75}$ (M1). ME was significantly lower on M4 than the other diets. ME was in the chicken experiment between 1513 (C2) and 1578 kJ/$kg^{0.75}$ (C4) and in the pig experiment between 1360 (P3) and 1442 kJ/$kg^{0.75}$ (P4). The difference between diets in the chicken and pig experiment was non-significant.

The HE was not affected by diet in the experiments. HE was between 619 (M2) and 644 kJ/kg$^{0.75}$ (M4) in the mink (paper A: Table V), 792 (C4) and 877 kJ/kg$^{0.75}$ (C3) in the chicken (paper B: Table 4) and 730 (P2) and 777 kJ/kg$^{0.75}$ (P4) in the pig (paper C: Table 5). HE was somewhat lower in mink than both the chicken and pig but the growth rate of the mink is also lower than both chicken and the pig. It was unexpected that the HE in the mink experiment on M4 not was affected by diet as the feed intake was decreased. In pigs a decreased feed intake led to a lower HE (Chwalibog et al., 1994). Even in period 1, where the intake of feed was reduced by 50% on M4 the HE was the same as on M1, M2 and M3. If HE is compared with the data for the protein turnover there could be an explanation, because the protein synthesis and breakdown increased with increasing dietary content of BPM, and this process costs energy. In the pig, where protein turnover also was measured, no differences in protein synthesis and breakdown were found with increasing dietary content of BPM.

The measured HE in the pig experiment was slightly lower than found for pigs weighing 20-40 kg (Chwalibog et al., 2005) but in agreement with Noblet et al. (2003). The HE in periods 2 and 3 in the pig experiment was in agreement with Chwalibog et al. (2005). HE values for the mink kits were slightly higher than those measured by Chwalibog et al. (1982). The values for the chickens are in agreement with Noblet et al. (2003). It may be concluded that the HE of the three species were in fair agreement with values found in the literature.

As ME and HE were the same on all diets in the chicken experiment (paper B: Table 4) and in the pig experiment (paper C: Table 5) the RE was also the same. In the mink the decreased level of ME on M4 led to lower RE on this diet. The RE was –11 kJ/kg$^{0.75}$ on M4, which indicates zero weight gain (paper A: Table V). As the RN was positive the fat retention was negative and the mink on M4 has mobilized energy. The non-protein respiratory quotient (RQ_{np}) was 0.75 on M4 and significantly lower than on M2 and M3, and the oxidation of fat (OXF) as a percentage of the total HE was also higher on this diet (paper A: Table V and Figure 3). The mink experiment was only a short-term study where experimental diets were fed during the five experimental periods. The animals on M4 had the same body weight gain as animals on the other diets between experimental periods (unpublished data), and it can be concluded that no irreversible adaptation of the metabolism occurred during the experimental periods. There were, however, differences in RE between the different balance periods. In periods 2 and 3, where also the feed intake based on

metabolic size was the same as for the control diet, RE was positive and nearly the same as on the other diets. In periods 1, 4 and 5 RE was negative.

In the chicken and pig no differences in oxidation pattern of nutrients were found. Respiratory quotient (RQ) in the chicken experiment (paper B: Table 4) and the RQ_{np} in the pig experiment (paper C: Table 5) was also unaffected by dietary treatment. The RE was between 613 $kJ/kg^{0.75}$ and 696 $kJ/kg^{0.75}$ in the pig and between 653 $kJ/kg^{0.75}$ and 786 $kJ/kg^{0.75}$ in the chicken, which was much higher than in the mink, which had a RE of 150 $kJ/kg^{0.75}$ (M1), 152 $kJ/kg^{0.75}$ (M2) and 162 $kJ/kg^{0.75}$ (M3).

The higher growth rate of chickens and pigs compared with the mink had a considerable effect on the nitrogen and energy metabolism data. Comparison on metabolic body size has shown that the chicken had the highest RN, ME, HE and RE and the mink had the lowest.

Carcass composition

The chemical composition of the carcasses was investigated in the chickens because a decrease in the dressing percent of chickens fed increasing levels of dietary BPM have been observed (Skrede et al., 2003). From the energy metabolism measurements it was shown that the fat retention was not affected by diet and the same was shown in carcasses of the chickens. Dry matter, nitrogen, fat and energy content were the same on all diets. The ash content was for some unknown reason higher on C2 and C4 than C3. In the pig experiment the animals had the same fat and nitrogen retention on all diets. In the mink experiment animals fed diets M1, M2 and M3 also had the same retention of nitrogen and fat.

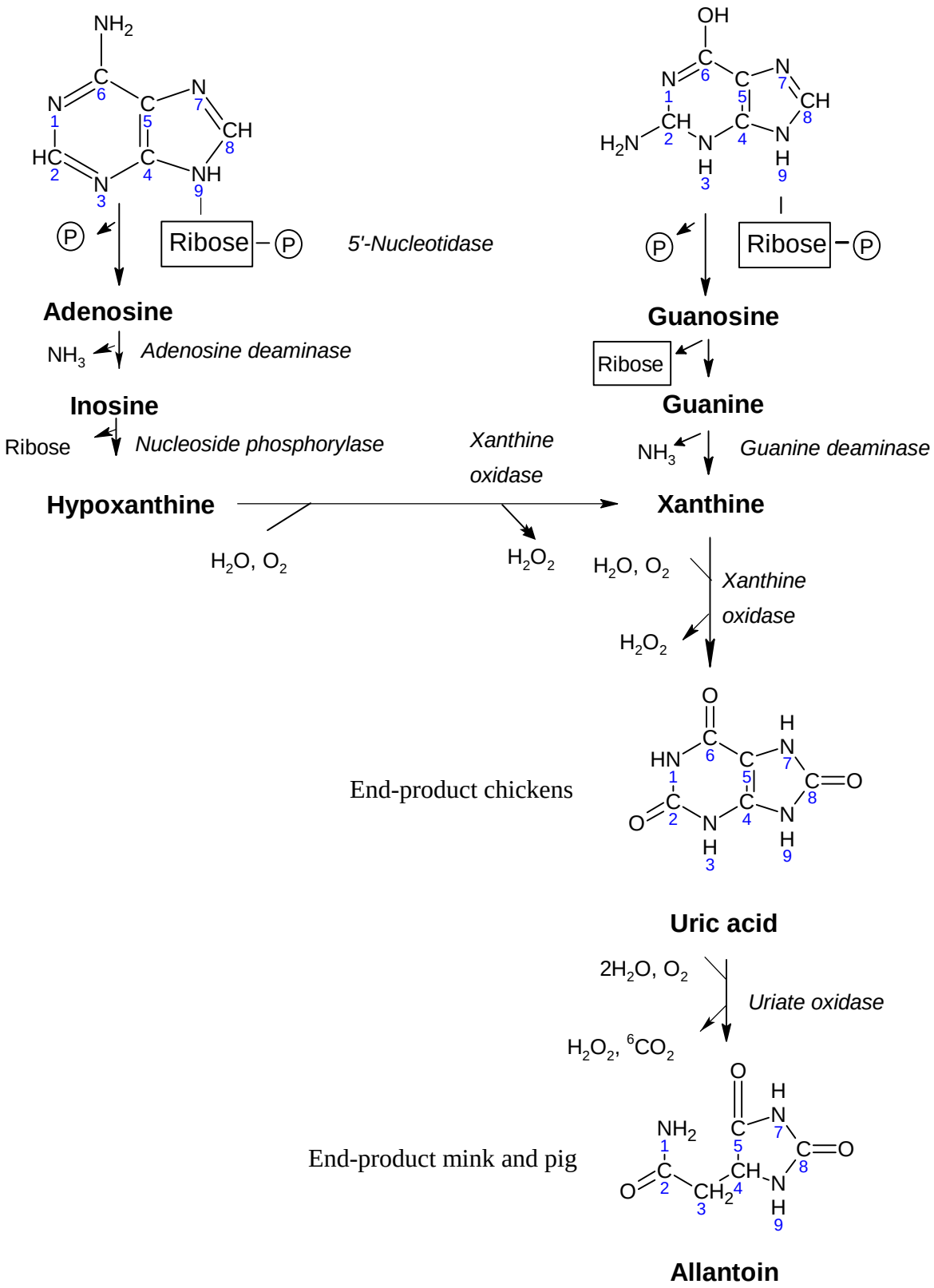

Figure 2. Decomposition of purine bases (modified after Greife 1984b and Michal, 1999)

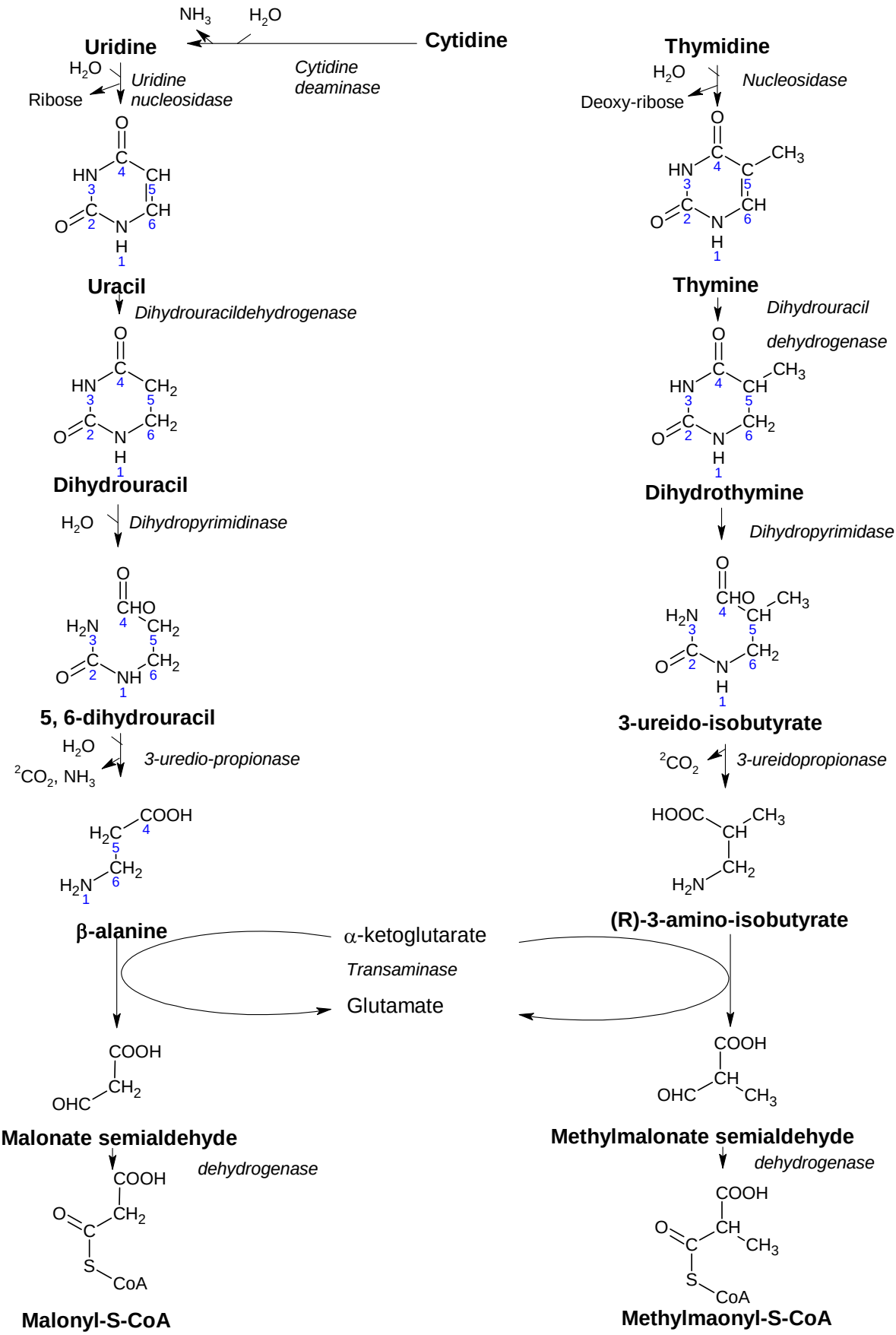

Figure 3. Decomposition of pyrimidine bases (modified after Greife 1984b and Michal, 1999)

Conclusion

From the three experiments with BPM to growing animals the following can be concluded:

- Mink kits on M4 had a significantly lower feed intake and this was probably because the diet was stickier than the other diets.
- In the mink experiment ADN, ADF and ADCHO decreased significantly with increasing BPM content. In the pig experiment only ADN decreased with increasing BPM content.
- The decrease in ADN was somewhat higher in the mink experiment than in the pig experiment. However, different control protein sources were used: in the mink experiment high quality fish meal was used as control protein, which may be expected to have a higher ADN than soybean meal which was used as control protein in pig experiment.
- The RN was not affected by diet neither in the mink nor the pig. In the chicken experiment the RN was slightly higher on C1 than the diets with BPM. This was probably caused by a higher protein content of C1.
- HE was not affected by diet neither in the mink, chicken nor the pig experiments.
- RE was positive on all experimental diets except mink fed diet M4, which had a RE at zero. The lower RE on M4 was a combination of lower feed intake, lower ADE and higher protein turnover.
- The RQ in the chicken experiment and RQ_{np} in the pig experiment were not affected by diet. RQ_{np} on M4 in the mink experiment was significantly lower than on M2 and M3.
- The oxidation of protein was significantly lower and OXF was significantly higher on M4 than the other diets in the mink experiment. The oxidation of nutrients was not affected by diet in the chicken and pig experiments.
- Increasing dietary content of BPM led to an increase in the protein turnover in the mink but not in the pig. The differences observed between the mink and pigs were probably related to composition of the diets.
- An increase in the intake of NAN led to an increase in the excretion of allantoin both in the mink and pig. There were, however, differences between the mink and pig. The mink excreted more purine base derivatives than purine bases ingested on all diets whereas the pigs on P2, P3 and P4 excreted less than ingested.

- In general, there was no interaction between diet and period, and it is concluded that BPM can be used to newly hatched birds and newly weaned mink and pigs without negative effects on their RN, HE and RE.
- The chicken had higher RN, ME, HE and RE than pig and mink. The differences between the pig and chicken were smaller than the differences between the mink and chicken.

From the experiments it is concluded that BPM has no adverse effects on the protein and energy metabolism in mink, chicken and pig as long as the N derived from BPM not exceeds 40%, 20% and 50% in the diets for the three animals, respectively.

Future research areas

The three experiments have shown that BPM is a high-quality protein feedstuff, which can supply up to 40%, 20% and 50% in the mink, chicken and pig, respectively. Although the feedstuff can be used as protein source for animals, there are a number of effects caused by BPM, which could be of scientific interest.

- To evaluate the decrease in feed intake in the mink experiment, when more than 40% of the nitrogen derived from BPM. The observation from the mink experiment has indicated that the consistency might be a problem.
- To evaluate the effect on the protein and energy metabolism in chickens, when more than 20% of the N derived from BPM. Furthermore the purine base metabolism could be of interest as chickens excreted all nitrogen as uric acid.
- Digestibility studies of BPM divided into different fractions i.e. cytoplasm, cell walls and internal membrane system to evaluate the impact on the different fractions on the total digestibility.
- To evaluate whether the increase in ADF in the pig experiment was related to an increase in ADF in BPM or if it was related to the increase in fat intake.
- To evaluate the digestibility of RNA and DNA and the retention of purine and pyrimidine bases in the mink.
- To evaluate the impact of BPM on the microbial fermentation in the intestine.
- To evaluate the impact of BPM on the development of the intestine after weaning, because dietary nucleotides probably play a roll in the growth and development of the intestine (Tsujinaka et al., 1999).
- To evaluate whether the increase in protein turnover in the mink experiment was caused by changes in the amino acid composition of diets with increasing dietary content of BPM.
- To investigate the decrease of urinary creatinine with increasing dietary content of BPM in mink.

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Abbreviations

ADCHO:	Apparent digestibility of carbohydrate
ADF:	Apparent digestibility of fat
ADN:	Apparent digestibility of nitrogen
AMP:	Adenosinemonophosphate
BPM:	Bacterial protein meal
DM:	Dry matter
DN:	Digested nitrogen
GMP:	Guanosinemonophosphate
HE:	Heat production
IN:	Intake of nitrogen
ME:	Metabolisable energy
NAN:	Nucleic acid nitrogen
NRC:	National Research Council
OXF:	Oxidation of fat
PHB:	Poly-β-hydroxybutyrate
RE:	Retained energy
RN:	Retained nitrogen
RQ:	Respiratory quotient
RQ_{np}:	Non-protein respiratory quotient
SBM:	Soy bean meal

The papers included in this thesis have all been published. The references to the published papers can be found below. Paper A is not included in this PDF.

Paper A:
Hellwing, A. L., Tauson, A.-H., Ahlstrøm, Ø., & Skrede, A. (2005). Nitrogen and energy balance in growing mink (Mustela vison) fed different levels of bacterial protein meal produced with natural gas. *Archives of Animal Nutrition , 59* (5), 335-352. DOI: 10.1080/17450390500247873

Paper B:
Hellwing, A. L., Tauson, A.-H., & Skrede, A. (2006). Effect of bacterial protein meal on protein and energy metabolism in growing chickens. *Archives of Animal Nutrition, 60* (5), 365-381. DOI: 10.1080/17450390600884351

Paper C:
Hellwing, A. L., Tauson, A.-H., Kjos, N. P., & Skrede, A. (2007). Bacterial protein meal in diets for growing pigs: effects on protein and energy metabolism. *Animal , 1* (1), 45-54. DOI: 10.1017/S1751731107283879

Paper D:
Hellwing, A. L., Tauson, A.-H., Skrede, A., Kjos, N. P., & Ahlstrøm, Ø. (2007). Bacterial protein meal in diets for pigs and mink - protein turnover and urinary excretion of purine derivatives. *Archives of Animal Nutrition , 61* (6), 425-443. DOI: 10.1080/17450390701565248

Effect of bacterial protein meal on protein and energy metabolism in growing chickens[1]

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Abbreviations key

BPM = Bacterial protein meal, GE = Gross energy, HE = Heat production, IN = Ingested nitrogen, ME = Metabolizable energy, NAN = Nucleic acid nitrogen, RE = Retained energy, RFE = Energy retained in fat, RN = Retained nitrogen, RPE = Energy retained in protein, UN = Urinary nitrogen, OXCHO = Oxidation of carbohydrate, OXF = Oxidation of fat.

[1] A preliminary report on the experiment was presented at the EAAP symposium held in Rostock-Warnemünde in September 2003, as follows: Hellwing, A.L.F., A-H. Tauson, and A. Chwalibog. 2003. Energy and nitrogen balance in slaughter chickens fed bacterial protein. Pages 179-182 in Progress in Research on Energy and Protein Metabolism, EAAP Publication No. 109, Wageningen Academic Publishers, Wageningen.

ABSTRACT This experiment investigates the effect of increasing the dietary content of bacterial protein meal (BPM) on the protein and energy metabolism, and carcass chemical composition of growing chickens. Seventy-two Ross male chickens were allocated to four diets, each in three replicates with 0% (D0), 2% (D2), 4% (D4), and 6% BPM (D6), BPM providing up to 20% of total dietary N. Five balance experiments were conducted when the chickens were 3–7, 10–14, 17–21, 23–27, and 30–34 days old. During the same periods, 22-hour respiration experiments (indirect calorimetry) were performed with groups of 6 chickens (period 1), 5 chickens (period 2), and 1 chicken (periods 3–5). After each balance period, one chicken in each cage was killed and the carcass weight was recorded. Chemical analyses were performed on the carcasses from periods 1, 3, and 5. Weight gain, feed intake, and feed conversion rate were found to be similar for all diets. Chickens on D0 retained 1.59 g N/kg$^{0.75}$/day, significantly more than chickens on D2, D4, and D6, which retained 1.44 g, 1.52 g, and 1.50 g N/kg$^{0.75}$/day, respectively. This was probably caused by the higher nitrogen content of D0. Neither the heat production (P = 0.92) nor the retention of energy (P = 0.88) were affected by diet. Carcass composition was similar between diets, in line with the values for protein and energy retention found in the balance and respiration experiments. It was concluded that the overall protein and energy metabolism as well as carcass composition were not influenced by a dietary content of up to 6% BPM corresponding to 20% of dietary N.

(*Key words:* chickens, bacterial protein meal, protein metabolism, energy metabolism, nucleic acids, carcass composition)

INTRODUCTION

In animal nutrition, bacterial protein meal (BPM) can provide an alternative protein source to fish meal and meat-and-bone meal, and a non-GMO alternative to gene-modified vegetable protein sources. It is produced by the continuous fermentation of natural gas and ammonia by *Methylococcus capsulatus* (Bath)(>90%), *Ralstonia* sp., *Brevibacillus agri*, and *Aneurinibacillus* sp. (Skrede *et al*., 1998). The bacterial culture is concentrated, heat sterilized, and spray dried to obtain a dry product with high storage stability. BPM is reddish/brown with about 96% dry matter (DM), 70% crude protein (CP), and 10% fat. The amino acid profile is similar to that of fish meal, except that the lysine content is somewhat lower and the tryptophan content higher (Skrede *et al*., 1998). BPM has a content of about 9.5% RNA and DNA, and as RNA and DNA contain 140 g N kg^{-1}, about 12% of the N in BPM is derived from nucleic acids. This level is considerably higher than that found in other feedstuffs, such as fish meal (Greife, 1984), but it is lower than is reported for other bacterial proteins (Braude *et al*., 1977; Kiessling and Askbrandt, 1993).

BPM has earlier been evaluated as a dietary protein source in production experiments with chickens (Skrede *et al*., 2003). Furthermore, BPM has been found to be a suitable protein source for pigs (Øverland *et al*., 2001, 2004), blue foxes (Skrede and Ahlstrøm, 2002), and Atlantic salmon (Storebakken *et al*., 2004; Berge *et al*., 2005).

Whether nucleic acid N (NAN) can be utilized or whether it is only excreted—a process that costs energy—is an issue of importance when evaluating BPM as a protein source. In earlier studies with chickens, the apparent digestibility of yeast RNA-N was determined to be 77.2% (Shannon and McNab 1972) and even as high as 87–95% (Greife and Molnar, 1980), depending on the RNA content. The latter study found that nitrogen retention was not affected by RNA-N content, provided the diets contained equal levels of amino acid N (Greife and Molnar, 1980); similar findings were reported for a combination of purine and pyrimidine bases (D'Mello, 1979; D'Mello, 1986). Yeast RNA added to a pig diet increased N retention, provided that the diet was limited in protein and the supply of essential amino acids was sufficient to satisfy requirements (Roth and Kirchgessner, 1977, 1978). These findings suggest that chickens may be able to utilize RNA-N, and possibly different combinations of purine and pyrimidine bases, as a source of non-specific N; therefore, it

could be speculated that a dietary BPM supply might be beneficial, provided there is an adequate essential amino acid supply.

Studies of the effects of methanol-grown bacterial protein on the retention of nitrogen (RN) and on the utilization of ingested nitrogen (IN) for retention (RN/IN) have produced conflicting results. Some studies have reported unchanged RN and RN/IN (D'Mello and Acamovic, 1976; Plavnik *et al*., 1981), while others found a reduction in RN/IN (D'Mello and Acamovic, 1976) when the dietary level of bacterial protein was 9–10%. D'Mello and Acamovic (1976) found that consumption of more than 10% methanol-grown bacterial protein reduced RN/IN and the N content, but increased the fat content, of carcasses.

The influence of BPM on RN, heat energy (HE), and retention of energy (RE) has hitherto only been investigated in mink. When feed intake was kept equal, increasing amounts of BPM in the diets of mink had no effects on RN, HE, and RE, although the digestibility of nitrogen, fat, and energy decreased with increasing dietary BPM levels (Hellwing *et al*., 2005). However, when dietary BPM supply made up 60% of N, feed intake was significantly reduced (Hellwing *et al*., 2005). Reduced feed intake, but improved feed conversion, has also been reported in chickens fed high levels of bacterial protein replacing conventional protein sources (Bornstein *et al*., 1981; Plavnik *et al*., 1981; Skrede *et al*., 2003).

The aim of the present study was to evaluate how increasing dietary levels of BPM, and hence increasing levels of nucleic acid N, affect protein and energy metabolism in young growing chickens.

MATERIALS AND METHODS

Animals and Experimental Design

Seventy-two-day-old male Ross chickens were bought from a commercial hatchery. At arrival, the chickens were weighed, individually marked, grouped according to weight, and then randomly allocated to four dietary treatment groups, each in three replicates (A, B, and C). The experiment was carried out in accordance with The Animal Experimentation Act in Denmark (law no. 726, September 9, 1993).

Experimental diets and water were provided *ad libitum* from day one. The experiment comprised five periods during which balance and 22-hour respiration experiments by means of indirect calorimetry in an open-air circulation system were carried out. All replicates were used in the balance experiments but only two replicates (A and B) were used in the respiration experiments (Table 1). The chickens were 3, 10, 17, 23, and 30 days old at the start of the five, 4-day balance periods. During balance periods 1 and 2, all chickens from a single cage were measured in the respiration experiment. From balance period 3 onwards, only one and always the same chicken from each cage was used, owing to the limited ventilation capacity of the respiration unit (Table 1).

The original number of animals per cage (six in the first balance period) was subsequently reduced by one randomly selected chicken per balance period, until two chickens remained in the fifth period.

Diets and Feeding Routines

The BPM[2] was derived from an experimental batch. One of the four experimental diets served as control and contained no BPM (D0), while the remaining three diets had dietary BPM contents of 2% (D2), 4% (D4), and 6% (D6). The BPM replaced fish meal on a crude protein basis, and crude protein derived from BPM made up 6.5%, 13.2%, and 19.9% of total dietary N in diets D2, D4, and D6, respectively. The diets were formulated so as to have equal levels of metabolizable energy and to be iso-nitrogenous, and were produced by the Center for Feed Technology, Ås, Norway. The

[2] The BPM used is of the trade name Bioprotein (Norferm AS, Stavanger, Norway).

diets were pelleted using a Münch pellet press[3] equipped with a 3-mm die. Dietary composition is given in Table 2 together with analyzed chemical composition. The contents of amino acids, and purine and pyrimidine bases are given in Table 3.

Housing

The chickens were housed in a 3-tier rack of twelve metabolic cages with four cages per tier. All diets were represented by one cage per tier. The cages were each 0.5 m × 0.5 m × 0.5 m in size. They had a plastic-coated wire floor, with a mesh size of 0.5 cm × 1 cm, located 7 cm above the bottom of the cages. During the respiration experiments, the chickens were placed in metabolic cages in the respiration chambers.

For the first 10 days after arrival the room temperature was kept at 27°C, and a heating lamp inside each cage ensured that the temperature in the cage was 30–33°C. Then over the next several weeks the room temperature was gradually lowered from 27°C to 22°C. A 23-hour light:1-hour dark light cycle was used.

Balance Experiment—Collection Procedures

Chickens were weighed on the first and last day of each balance period. Collection procedures were performed daily between 9:00 and 12:00 a.m. Feed residues and droppings were weighed and frozen at –18°C after collection. Animals allocated to respiration experiments were brought to the respiration unit between 9.00 a.m. and 10 a.m. The chickens were placed in the metabolic cages used for the respiration experiments and given water and a weighed amount of feed. The next day the animals were brought back, and droppings and feed residues were collected. At the end of each balance period, droppings were homogenized, sampled for analyses of wet material, and the rest was freeze-dried.

[3] Münch-Edelstahl GmbH, Hilden, Germany.

Respiration Experiment

The measurements started at 11.00 a.m. and ended the following day at 9.00 a.m. The respiration chambers each had a volume of 760 L, and were constructed for animals with a live weight of 0.5 to 5 kg. For a detailed description of the calibration and measurement procedures, see Chwalibog *et al*. (2004).

Carcass Sample Collection

The chickens selected for carcass analyses were fasted overnight and killed by means of CO_2. The carcasses were weighed and frozen at –18°C for later analyses. The chickens from periods 1, 3, and 5 were analyzed for DM, ash, N, fat, and gross energy (GE) after removal of the livers.

Analytical Procedures

All diets in all periods were analyzed for DM, ash, N, fat, and GE. Dropping samples were mixed carefully before being sampled for the analyses of DM and N. The N content was analyzed using wet material except in period 1, when it was analyzed using freeze-dried material. The freeze-dried droppings were milled, mixed to homogeneity, and then analyzed for DM, ash, fat, and GE.

The carcasses were taken out of the freezer and then chopped twice in a mincing machine[4] before being mixed to homogeneity. Sampling of material for analyses was as described above.

DM was determined by evaporation at 105°C to constant weight. Ash was determined by combustion at 525 C°. N was determined by means of the micro-Kjeldahl technique using the Tecator-Kjeltec system 1030[5]. Crude protein (CP) was calculated as N × 6.25. Fat was determined by petroleum ether extraction in a Soxtec system after HCl hydrolysis. GE was determined using an adiabatic bomb calorimeter[6]. The amino acids, except tryptophan, in the diets were determined according to the European Community Directive 98/64/EC (OJ 1998). Tryptophan was analyzed according to the procedure of Bech-Andersen (1991); for a more detailed description of the method,

[4] BIZERBA, Bizerba-Werks Wilhelm Kraut GmbH&Co, Hamburg, Germany
[5] Tecator AB, Höganäs, Sweden
[6] IKA-Calorimeter system, IKA®Gmbh & Co. KG, Staufen, Germany

see Skrede *et al*. (2003). An HPLC procedure was used to determine the contents of adenine, guanine, thymine, uracil, and cytosine, as described in detail by Thode (1999).

Calculations

Carbohydrates (CHO) were calculated by difference. RN was calculated as N intake minus N in droppings. Metabolizable energy (ME) was calculated as ME, kcal = GE in feed – energy in droppings. HE was calculated according to Brouwer (1965) as HE, kcal = 3.886 × O_2, L + 1.200 × CO_2, L – 1.431 × UN, g, but without correction for UN because the content of uric acid in droppings was not analyzed. RE, kcal was calculated as ME – HE. Oxidation of carbohydrate (OXCHO) and fat (OXF) were calculated according to the equations given by Chwalibog *et al*. (1992).

Statistical Analyses

Statistical analyses of data from the balance and respiration experiments were carried out by means of the MIXED Procedure in SAS®[7] (Littell *et al*., 1996), using the following model:

$$Y_{ijk} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \varepsilon_{ijk}$$

where Y_{ijk} is the Y_{ijk}*th* observation, μ is the general mean, α_i is the fixed effect of diet (D0, D2, D4, and D6), β_j is the fixed effect of balance period (1 to 5), $\alpha\beta_{ij}$ is the interaction between diet and balance period, and ε_{ijk} is the residual error. Results were analyzed as repeated measurements, and the autoregressive order 1 (AR(1)) covariance structure was fitted (Littell *et al*., 1996). Results are presented as least squares means (LSmeans), and the square root of residuals (RR) is given for each variable. Pair-wise comparisons of LSmeans were made using the PDIFF option, and effects were considered significant if $P < 0.05$. Data from one cage in period 2 was omitted from analyses of the balance experiment data because of technical problems, but not from the respiration experiment.

[7] SAS for Windows, 1998, version 8 edition, SAS Institute INC., Cary, NC

Statistical analyses of body weight and of the chemical composition of carcasses were all performed using the general linear model (GLM) procedures included in SAS® (SAS Institute Inc., 1990), with the same model as was used in the balance and respiration experiments. Results are presented as LSmeans and the root mean square error (RMSE) is given as a measure of variance. Two chickens died during balance period 3 for reasons unrelated to the experimental treatment and were thus omitted from data analysis.

RESULTS

All results regarding nutrient intake and energy metabolism are presented in relation to metabolic body size ($kg^{0.75}$) in order to facilitate comparisons among balance periods. Results regarding intake of amino acids are presented in g per day, and those regarding intake of purine and pyrimidine bases in mg per day. All results are presented per chicken per day.

Intake of Nutrients and Performance

Effect of Diet. The DM intake was highest on D6 with 111 $g/kg^{0.75}$ and lowest on D2 with 105 $g/kg^{0.75}$. Although the difference in DM intake was small, there was a tendency for a significant diet effect ($P = 0.07$) (Table 4). The contents of crude protein, fat, and CHO in the diets differed somewhat, resulting in significant differences in the intake of protein, fat, and CHO among the diets. Chickens on D2, D4, and D6 had the same intake of crude protein, which was significantly lower than that of the control diet. The intake of fat increased significantly from D0 to D6, whereas the intake of CHO on D0 and D2 was similar and significantly lower than on D4 and D6 (Table 4). The intake of GE differed by 27 $kcal/kg^{0.75}$ between D2 and D6, and although the difference was numerically small the GE intake on D0 and D2 was significantly lower than on D6 ($P = 0.02$). The intakes of lysine, methionine, methionine plus cystine, and threonine were higher on D0 than on D6, whereas the intake of tryptophan was higher on D6 than on the other diets (Table 4). The intake of adenine, cytosine, and uracil increased with increasing dietary content of BPM. Intake of thymine was significantly higher on D4 than on D0 and D2. The intake of guanine was the same on all diets (Table 4). Despite the reported difference in nutrient intake, the daily weight gain, feed intake, and feed conversion rate were not affected by diet.

Effect of Period. The daily weight gain and feed intake increased significantly over the five balance periods. The feed conversion rate was significantly better in periods 1, 2, and 3 than in period 4, which again was better than in period 5. Intake of nutrients in relation to metabolic body size was highest in period 2. The intakes of DM, protein, fat, and GE were significantly higher in period 2 than in period 1. In periods 3, 4, and 5 the intake of nutrients was significantly lower than in the previous period (Table 4). The intake of amino acids, and of purine and pyrimidine bases increased significantly from period 1 to period 5.

Protein and Energy Metabolism

Effect of Diet. The intake of NAN increased from 0.13 g/kg$^{0.75}$ on D0 to 0.19 g/kg$^{0.75}$ on D6, but whether including or excluding NAN, the intake of nitrogen was significantly higher on D0 than on the other diets (Table 5). The higher intake of nitrogen on D0 caused a tendency towards a higher excretion of nitrogen (P = 0.09) and a significantly higher RN (P = 0.01). The utilization of IN for RN (RN/IN) did not differ significantly between diets, neither when related to total IN nor when NAN was omitted. ME, HE, RE, energy retained in protein (RPE), energy retained in fat (RFE), and the respiratory quotient (RQ) were not significantly affected by diet (Table 5), and neither were OXCHO nor OXF (Figure 1a).

Effect of Period. RN was highest in periods 1 and 2, thereafter decreasing significantly (Table 5); RN/IN decreased significantly from period 1 to period 5. Both ME and HE increased from periods 1 to 2 and then decreased. RE decreased from 204 kcal/kg$^{0.75}$ in period 1 to 139 kcal/kg$^{0.75}$ in period 5, and the amount of energy retained in protein was always higher than the amount of energy retained as fat. RFE decreased by 30% from periods 1 to 5, but the difference was not significant. RQ was close to 1.00 in periods 1 and 2, but decreased to approximately 0.9 in periods 3, 4, and 5. The decrease in RQ and the great variation in HE, RE, and RFE were probably caused by the reduced feed intake of one chicken during the respiration experiment in periods 3, 4, and 5 compared with the feed intake during the balance experiment, and by a great variation between animals on the same diet (data not shown). OXF was significantly lower in period 1 than in periods 3, 4, and 5; consequently, OXCHO was significantly higher in period 1 than in periods 3, 4, and 5 (Figure 1b).

Interaction between Diet and Period. The ranking of the different diets in the different periods with respect to RN, RN/IN, and RN/(IN-NAN) were not the same, and this caused significant interaction effects, *e.g.* chickens on D0 in period 1 had the highest level for these traits whereas the same chickens had the lowest level in period 2.

Chemical Composition of Carcasses

Effect of Diet. The weight of the carcasses was not significantly affected by diet ($P = 0.09$). The carcass contents of DM, N, fat, and gross energy were not affected by treatment, but the ash content was significantly lower on D4 than on D2 and D6 (Table 6).

Effect of Period. The carcass ash content was the same in the three periods (1, 3, and 5). The DM and N contents increased significantly from periods 1 to 3 as well as from periods 3 to 5. The fat and energy contents increased significantly from periods 1 to 3, but not from periods 3 to 5.

DISCUSSION

Protein and Energy Metabolism

Protein and energy metabolism traits were generally found to be unaffected by dietary treatment in the present study, the exception being RN, which was lower on diets containing BPM than on the control diet. This was likely caused by lower dietary crude protein content and thus a lower intake of crude protein, as well as likely by the limiting amino acids, *e.i.* lysine, methionine, methionine and cystine, and threonine, among chickens fed diets containing BPM. Diets containing different levels of BPM showed small differences in crude protein content, but resulted in similar RN values, although the contents of methionine, methionine plus cystine, and threonine, especially on D4, were slightly below the minimum requirements specified by NRC (1994). Our results suggest that a dietary supply of BPM up to 6% (approximately 20% of dietary N), replacing fish meal N, supported normal performance, protein retention, and energy metabolism. A well-maintained RN level has been reported for chickens fed 9% methanol-grown bacterial protein (Plavnik *et al*., 1981), as well as for chickens fed up to 5% yeast RNA (Greife and Molnar, 1980)

Because we were working with intact birds, it was obviously impossible to investigate the effect of BPM consumption on the excretion pattern of nucleic acid or nucleic acid derivatives in urine and feces in chickens. It has previously been shown that the digestibility of yeast RNA is high in chickens (Greife and Molnar, 1980), and it may be assumed that most of the NAN in the chicken diets was digested. Some of the digested NAN is probably directly deposited in the body as nucleic acid in RNA and DNA (Greife and Molnar, 1984a, b). Nitrogen from purine and pyrimidine bases may also provide a source of non-specific N for the synthesis of non-essential amino acids, and thus contribute to the N retention in animals fed diets with a low N content but a sufficient content of essential amino acids (D'Mello, 1979). In the present study, the efficiency of N retention (RN/IN) was the same on all diets, despite the higher RN on the control diet without BPM. If NAN was omitted from IN, the efficiency of N retention increased, especially on the diet with the highest level of BPM, thus indicating a slightly higher utilization of amino acid N than of nucleic acid N. Because of our experimented approach, however, it was impossible to evaluate whether nucleic acids were retained or utilized for the synthesis of non-essential amino acids, nor was it an objective of the study.

Both total nucleic acid content and the relative proportions of the individual purine and pyrimidine bases may influence chicken growth: an inclusion of 0.1% free adenine supported normal feed intake and body weight gain, whereas feed intake and growth were negatively affected when free adenine made up about 1% of the diet (Baker and Molitoris, 1974; D'Mello, 1986). Furthermore, studies in rats have shown that only free adenine caused depression in feed intake and growth, whereas both adenosine and adenylate support normal growth (Brulé *et al*., 1988). The total content of adenine in our diets did not exceed 0.1%, so the inclusion of 6% BPM in the diet was below the level where free adenine may cause adverse effects.

The lower RN of chickens fed BPM, shown in the present study, and the interaction observed between diets and periods for both RN and RN/IN may be explained by differences in the levels of digestible protein and amino acids among diets. As shown in Table 2, the crude protein level was lower in the BPM-containing diets than in the control diet. Furthermore, the amino acid digestibility of BPM in the chicken (especially of the cysteine contained therein) is lower than that of fish meal (Skrede *et al*., 1998), and increasing substitution of fish meal with BPM would be expected to reduce the digestibility of most amino acids. In addition, the contents of methionine, methionine plus cystine, and threonine on D4 and the content of methionine plus cystine on D6 were slightly lower than the minimum requirements for chickens between 0 and 3 weeks of age (NRC, 1994). This would be expected to affect N retention in young chickens more than in older birds, since the amino acid requirements are highest during early growth (NRC, 1994). This is reflected in the practical recommendation for the amino acid composition to be fed to Ross broilers, which is higher for the first 10 days than later on (Ross, 2002). In the first balance period, all limiting amino acids were lower than recommended for Ross broilers, except for methionine on D0 and D2, and isoleucine and tryptophan were lower than recommended on D6. In the second, third, and fourth balance periods only threonine on D2, D4, D6 and methionine plus cysteine on all diets differed from the recommendations. The deviation from the Ross recommendations was greatest on D4 and D6. This may explain the diet:period interaction shown for some parameters in the present study.

Social facilitation may have an influence on the eating behavior of chickens: chickens without visual contact with other chickens eat less than those, which have such contact (Keeling and Hurnik, 1996). This may explain the reduced feed intake of the single chickens that were allocated

to the respiration experiments from period 3 onwards, compared with that of the chickens only used in the balance experiments. The reduced feed intake influenced HE, RE, RFE, and RQ, but the intake during the respiration experiments from period 3 onwards was the same for all diets; therefore, comparisons between diets should be considered as valid, whereas comparisons between the first two and the last three balance periods should be regarded cautiously.

HE in chickens was unaffected by dietary BPM supply, as was also found in studies of mink kits fed different levels of BPM (Hellwing *et al*., 2005). The digested purine and pyrimidine bases may save energy if they are directly incorporated into RNA and DNA in the body, but if they are excreted, the decomposition requires O_2 and increases the total excretion of CO_2. A theoretical calculation of this cost was made, assuming that the purine and pyrimidine bases were 100% digestible and that purine bases were decomposed to uric acid and pyrimidine bases to their specific end products. If the consumption of O_2 and excretion of CO_2 related to these processes were subtracted, the recalculated HE differed less than 1% from the value reported in Table 5. Hence, the energy cost of decomposition of the nucleic acids was almost negligible compared to other metabolic processes in the body.

The fact that neither RE nor RQ were affected by dietary level of BPM conforms with previous findings regarding mink (Hellwing *et al*., 2005), provided the BPM used in that study accounted for a similar amount of dietary N as in the present study. In mink (Hellwing *et al*., 2005), but not in chickens, the pattern of substrate oxidation was affected by level of dietary BPM. This discrepancy between the studies can be explained by only OXF and OXCHO being calculated for chickens, whereas the mink data also encompassed oxidation of protein.

Changes in protein and energy intake, HE, RN, RE, and RN/IN over time showed the same pattern as reported by Chwalibog *et al*. (1985), but intake, retention, and utilization were all higher than the values of Chwalibog *et al*. (1985). The difference between our results and those of Chwalibog *et al*. (1985) may be an effect of different genotypes of chickens, since body gain and feed utilization of broiler chickens have improved greatly over the last 20 years.

Chemical Composition of the Carcasses

A previous study by Skrede *et al*. (2003) showed lower abdominal fat content in chickens fed 6 and 9% BPM, compared to the fat content achieved on a control diet devoid of BPM. The present study found no significant effect of diet on the fat content in carcasses, although the diets used had an increasing content of fat with an increasing content of BPM. The analyzed body composition agreed well with the protein and energy retention measured in the balance and respiration experiments. A previous study with a methanol-derived bacterial protein showed a reduction in nitrogen content and an increase in energy content of the body with increasing dietary levels of bacterial protein (D'Mello and Acamovic, 1976), but the levels of bacterial protein in diets used in that experiment were higher and the feed intake was also reduced on the two diets with the highest inclusion of bacterial protein. Hence, our results suggest that dietary BPM did not affect chemical body composition.

Conclusion

Animal performance and health, and nitrogen and energy metabolism traits were generally not affected by replacing high-quality fish meal with BPM comprising up to 6% of the diet—corresponding to 20% of the dietary N—in diets for broiler chickens from hatching until 35 days of age. Higher nitrogen retention in the control group was explained by a higher CP content and hence higher intake of amino acids on the control diet. Carcass composition was independent of diet and concurred with the retention of protein and energy measured in the balance and respiration experiments.

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TABLE 1. Number of animals per cage in each balance and respiration experiment. Only replicates A and B were used in the respiration experiments

			Period				
			1	2	3	4	5
	Age (days)		3–7	10–14	17–21	23–27	31–35
Diet	Replicates	Experiment					
D0	A	Balance/Respiration	6/6	5/5	4/1	3/1	2/1
	B	Balance/Respiration	6/6	5/5	4/1	3/1	2/1
	C	Balance	6	5	4	3	2
D2	A	Balance/Respiration	6/6	5/5	4/1	3/1	2/1
	B	Balance/Respiration	6/6	5/5	4/1	3/1	2/1
	C	Balance	6	5	4	3	2
D4	A	Balance/Respiration	6/6	5/5	4/1	3/1	2/1
	B	Balance/Respiration	6/6	5/5	4/1	3/1	2/1
	C	Balance	6	5	4	3	2
D6	A	Balance/Respiration	6/6	5/5	4/1	3/1	2/1
	B	Balance/Respiration	6/6	5/5	4/1	3/1	2/1
	C	Balance	6	5	4	3	2
Total	Balance		72	60	48	36	24
Total	Respiration		48	40	8	8	8

TABLE 2. Formulas and chemical composition of diets (%), and gross energy (GE) and metabolizable energy (ME) as Mcal/kg

	D0	D2	D4	D6
Ingredients				
Bacterial protein meal[1]	0	2	4	6
Fish meal[2]	6	4	2	0
Soybean meal[3]	20	20	20	20
Oats	19.2	19.1	19.0	18.6
Wheat	26	25.9	25.9	26
Maize	22	22	21.9	22
Soybean Oil	3	3.2	3.4	3.6
Limestone	1.1	1.1	1.1	1.1
Monocalcium phosphate	0.8	0.8	0.8	0.8
Sodium chloride	0.07	0.07	0.07	0.07
Sodium bicarbonate	0.25	0.25	0.25	0.25
Manganese oxide	0.01	0.01	0.01	0.01
Micro mineral premix[4]	0.15	0.15	0.15	0.15
Vitamin premix[5]	0.21	0.21	0.21	0.21
L-Lysine, HCl	0.3	0.3	0.3	0.3
DL-Methionine	0.2	0.2	0.2	0.2
Choline chloride	0.03	0.03	0.03	0.03
Betaine	0.08	0.08	0.08	0.08
Enzyme[6]	0.1	0.1	0.1	0.1
Titanium dioxide	0.5	0.5	0.5	0.5
Chemical composition				
Dry matter	90.3	90.3	90.4	90.6
Organic matter	83.9	84.2	84.7	85.1
N	3.7	3.5	3.4	3.4
Protein (N*6.25)	23.0	21.7	21.2	21.1
Carbohydrate	53.8	55.2	56.1	56.3
Fat	3.5	3.8	3.9	4.3
GE	4.1	4.2	4.2	4.2
ME[7]	3.1	3.1	3.1	3.0

[1] Norferm DA, Stavanger, Norway.
[2] Norseamink, Norsildmel, Bergen, Norway.
[3] Solvent extracted, not dehulled, Denofa AS, Fredrikstad, Norway.
[4] Mineral premix providing the following per kg feed: Fe 75 mg, Mn 60 mg, Zn 105 mg, Cu 15 mg, I 0.7 mg, Se 0.3 mg. Norferm.
[5] Vitamin premix providing the following per kg: vitamin A 5200 IU, vitamin D3 260 IU, dl-alfa-tocopheryl acetate 29 mg, menadione 4.2 mg, thiamine 1.6 mg, riboflavin 7.9 mg, pyridoxine 2.6 mg, d-pantothenic acid 9.5 mg, niacin 27.5 mg, biotin 0.12 mg, folic acid 1.45 mg, cyanocobalamine 0.01 mg.
[6] Avizyme 1200. Finnfeeds International, Marlborough, UK. The product contained 100 U/g β-glucanase and 2500 U/g xylanase extracted from *Trichoderma longibrachiatum*, and 800 U/g protease extracted from *Bacillus subtilis*.
[7] Calculated from the experimental data.

TABLE 3. Content of amino acids in the diets, g kg^{-1}, and purine and pyrimidine bases in mg kg^{-1}

Diet code	D0	D2	D4	D6
Essential amino acids				
Methionine	5.8	5.7	4.6	4.8
Threonine	7.9	7.7	6.9	7.5
Valine	10.3	10.3	9.8	10.8
Isoleucine	9.6	9.1	9.2	9.5
Leucine	16.6	15.9	16.3	15.6
Phenylalanine	11.1	10.5	10.7	9.9
Histidine	5.2	5.1	5.0	4.8
Lysine	13.8	13.5	12.6	12.2
Arginine	12.8	13.1	12.4	12.5
Tryptophan	2.2	2.2	2.3	2.6
Non-essential amino acids				
Cysteine	3.4	3.4	3.1	3.1
Tyrosine	7.5	8.1	7.5	7.2
Aspartic acid	19.7	19.2	17.3	18.7
Serine	10.4	10.4	9.6	10.0
Glutamic acid	40.0	38.4	35.7	38.5
Proline	11.7	11.2	11.1	12.1
Glycine	9.9	9.3	8.7	9.0
Alanine	10.1	10.2	8.6	9.8
Purine bases				
Adenine	449	643	767	949
Guanine	1400	1395	1442	1463
Pyrimidine bases				
Cytosine	497	668	812	956
Uracil	764	816	960	1099
Thymine	199	207	248	226
% N from purine and pyrimidine bases	2.9	3.6	4.1	4.6

TABLE 4. Intake of nutrients, purine and pyrimidine bases, and performance. Intake of bioprotein meal (BPM), dry matter (DM), crude protein (CP), fat, and carbohydrate (CHO) is given as g/kg$^{0.75}$ per day, gross energy (GE) as Kcal/kg$^{0.75}$ per day, amino acids as g/day, purine and pyrimidine bases as mg/day. Weight of animals, daily weight gain, and feed intake are given in g, and feed conversion rate in kg DM/kg gain.

	Diet				Period						*P*-value		
	D0	D2	D4	D6	1	2	3	4	5	RR[1]	Diet (D)	Period (P)	D * P
Age (days)					3–7	10–14	17–21	23–27	30–34				
Number of observations (n)	15	15	14	15	12	11	12	12	12				
BPM intake	0.0^{d}	2.3^{c}	4.8^{b}	7.4^{a}	3.9^{AB}	4.1^{A}	3.8^{B}	3.4^{C}	3.0^{D}	0.21	<0.001	<0.001	<0.001
DM intake	107	105	109	111	118^{B}	122^{A}	113^{C}	100^{D}	88^{E}	4.8	0.07	<0.001	0.82
CP intake (N*6.25)	27.2^{a}	25.5^{b}	25.6^{b}	25.9^{b}	28.3^{B}	29.3^{A}	27.2^{C}	24.1^{D}	21.3^{E}	1.1	0.01	<0.001	0.78
Fat intake	4.2^{d}	4.4^{c}	4.6^{b}	5.0^{a}	4.6^{C}	5.2^{A}	4.9^{B}	4.3^{D}	3.8^{E}	0.2	<0.001	<0.001	<0.001
CHO intake	68^{b}	68.9^{b}	71.9^{a}	73.3^{a}	77.1^{A}	79.3^{A}	73.7^{B}	65.1^{C}	57.6^{D}	3.1	<0.001	<0.001	0.87
GE intake	491^{b}	489^{b}	505^{ab}	516^{a}	544^{B}	563^{A}	523^{C}	462^{D}	409^{E}	22	0.02	<0.001	0.82
Lysine	1.43^{a}	1.36^{ab}	1.27^{b}	1.25^{b}	0.34^{E}	0.83^{D}	1.38^{C}	1.86^{B}	2.22^{A}	0.10	0.03	<0.001	0.56
Methionine	0.60^{a}	0.57^{a}	0.46^{b}	0.49^{b}	0.14^{E}	0.33^{D}	0.55^{C}	0.74^{B}	0.89^{A}	0.04	<0.001	<0.001	0.02
Methionine + cystine	0.95^{a}	0.91^{a}	0.77^{b}	0.80^{b}	0.22^{E}	0.54^{D}	0.90^{C}	1.21^{B}	1.43^{A}	0.06	<0.001	<0.001	0.11
Threonine	0.82^{a}	0.77^{a}	0.69^{b}	0.77^{ab}	0.20^{E}	0.48^{D}	0.79^{C}	1.07^{B}	1.27^{A}	0.06	0.03	<0.001	0.53
Tryptophan	0.23^{b}	0.23^{b}	0.23^{b}	0.26^{a}	0.06^{E}	0.15^{D}	0.25^{C}	0.33^{B}	0.40^{A}	0.02	0.03	<0.001	0.18
Adenine	38.7^{d}	53.5^{c}	63.8^{b}	80.8^{a}	15.1^{E}	37.1^{D}	61.5^{C}	83.2^{B}	99.0^{A}	4.8	<0.001	<0.001	<0.001
Guanine	120.5	116.2	120.0	124.6	30.9^{E}	75.6^{D}	125.4^{C}	168.9^{B}	201.0^{A}	9.1	0.56	<0.001	0.68
Cytosine	42.8^{d}	55.6^{c}	67.6^{b}	81.3^{a}	15.8^{E}	38.8^{D}	64.3^{C}	86.9^{B}	103.4^{A}	5.0	<0.001	<0.001	<0.001
Uracil	65.8^{c}	73.0^{bc}	79.9^{b}	93.5^{a}	20.0^{E}	49.0^{D}	81.2^{C}	109.6^{B}	130.4^{A}	6.1	<0.001	<0.001	<0.001
Thymine	17.2^{b}	17.2^{b}	20.6^{a}	19.3^{a}	4.8^{E}	11.7^{D}	19.3^{C}	26.0^{B}	31.0^{A}	1.4	0.003	<0.001	0.02
Weight	998	978	924	934	118^{E}	368^{D}	805^{C}	1408^{B}	2093^{A}	77	0.49	<0.001	0.89
Daily gain	69.2	68.9	66.2	67.2	20.4^{E}	48.7^{D}	80.4^{C}	91.1^{B}	98.9^{A}	6.5	0.74	<0.001	0.99
Feed intake	104	101	100	102	26^{E}	64^{D}	106^{C}	143^{B}	170^{A}	7.7	0.89	<0.001	0.87
Feed conversion rate	1.29	1.28	1.33	1.33	1.17^{C}	1.19^{C}	1.20^{C}	1.42^{B}	1.56^{A}	0.07	0.27	<0.001	0.48

[1] Residual error.

[a,b,c,d] Values with different superscripts differ significantly ($P < 0.05$).

[A,B,C,D,E] Values with different superscripts differ significantly ($P < 0.05$).

TABLE 5. Nitrogen and pro tein metabolism. Intake of total N (IN), intake of N without nucleic acid N (IN-NAN), excretion of N in droppings, retained nitrogen (RN), metabolizable energy (ME), heat energy (HE), retained energy (RE), energy retained as protein (RPE), and energy retained as fat (RFE) in Kcal/kg$^{0.75}$; respiratory quotient (RQ).

	Diet				Period						*P*-value		
	D0	D2	D4	D6	1	2	3	4	5	RR[1]	Diet (D)	Period (P)	D * P
Age (days)					3–7	10–14	17–21	23–27	30–34				
Number of observations in balance/respiration experiments (n)	15/10	15/10	14/10	15/10	12/8	11/8	12/8	12/8	12/8				
IN	4.35^{a}	4.07^{b}	4.10^{b}	4.14^{b}	4.53^{B}	4.69^{A}	4.35^{C}	3.85^{D}	3.40^{E}	0.18	0.01	<0.001	0.80
IN-NAN	4.22^{a}	3.93^{b}	3.93^{b}	3.95^{b}	4.36^{B}	4.51^{A}	4.19^{C}	3.70^{D}	3.28^{E}	0.18	0.001	<0.001	0.77
N in droppings	1.59	1.44	1.52	1.50	1.49^{BC}	1.62^{A}	1.55^{B}	1.48^{C}	1.41^{D}	0.10	0.09	<0.001	0.05
RN	2.75^{a}	2.63^{b}	2.58^{b}	2.64^{b}	3.04^{A}	3.06^{A}	2.81^{B}	2.37^{C}	1.99^{D}	0.13	0.01	<0.001	0.02
RN/IN	63.0	64.3	62.6	63.4	66.9^{A}	65.3^{B}	64.5^{B}	61.5^{C}	58.5^{D}	1.64	0.25	<0.001	0.002
RN/(IN-NAN)	64.9	66.7	65.3	66.5	69.6^{A}	67.8^{B}	67.0^{B}	63.9^{C}	60.8^{D}	1.7	0.15	<0.001	0.002
ME	364	362	374	377	393^{B}	420^{A}	393^{B}	337^{C}	303^{D}	20	0.39	<0.001	0.85
HE	190	205	210	189	189^{BC}	230^{A}	221^{A}	190^{B}	163^{C}	43	0.92	<0.001	0.89
RE	174	156	168	188	204^{A}	195^{AB}	172^{B}	147^{C}	140^{C}	47	0.88	0.03	0.42
RPE	98	94	93	94	108^{A}	110^{A}	100^{A}	84^{B}	71^{C}	5.8	0.23	<0.001	0.29
RFE	76	62	76	94	96	86	72	62	69	46	0.89	0.29	0.49
RQ	0.92	0.94	0.94	0.93	0.99^{A}	0.96^{AB}	0.89^{CD}	0.89^{D}	0.92^{BC}	0.05	0.90	<0.001	0.62

[1] Residual error.

^{a,b} Values with different superscripts differ significantly ($P < 0.05$).

^{A,B,C,D,E} Values with different superscripts differ significantly ($P < 0.05$).

TABLE 6. Average body weight (g) and chemical composition of carcasses. DM, ash, N, and fat are given in %, and gross energy (GE) in kcal/kg. Only chickens from periods 1, 3, and 5 were used for carcass analysis.

	Diet				Period						*P*-value		
	D0	D2	D4	D6	1	2	3	4	5	RMSE[1]	Diet (D)	Period (P)	D * P
Age (days)					3–7	10–14	17–21	23–27	30–34				
Number of observations (n)	17	17	18	18	12	12	10	12	24				
Body weight	1295	1248	1187	1224	235^{E}	561^{D}	1178^{C}	1747^{B}	2472^{A}	119	0.10	<0.001	0.86
Chemical body composition													
Number of chickens (n)	8	8	9	9	12		10		12				
DM	31.8	31.8	31.1	32.1	28.8^{C}		32.4^{B}		34.0^{A}	1.1	0.33	<0001	0.68
Ash	2.6^{ab}	2.7^{a}	2.5^{b}	2.7^{a}	2.6		2.7		2.6	0.1	0.04	0.18	0.69
N	3.0	2.9	3.0	2.9	2.7^{C}		3.0^{B}		3.1^{A}	0.1	0.39	<0.001	0.33
Fat	9.8	10.3	10.1	10.7	8.9^{B}		10.4^{A}		11.4^{A}	1.2	0.54	<0.001	0.48
GE	2011	2014	2006	2027	1750^{B}		2096^{A}		2197^{A}	114	0.98	<0.001	0.63

[1] root mean square error.
[a,b] Values with different superscripts differ significantly ($P < 0.05$).
[A,B,C,D,E] Values with different superscripts differ significantly ($P < 0.05$).

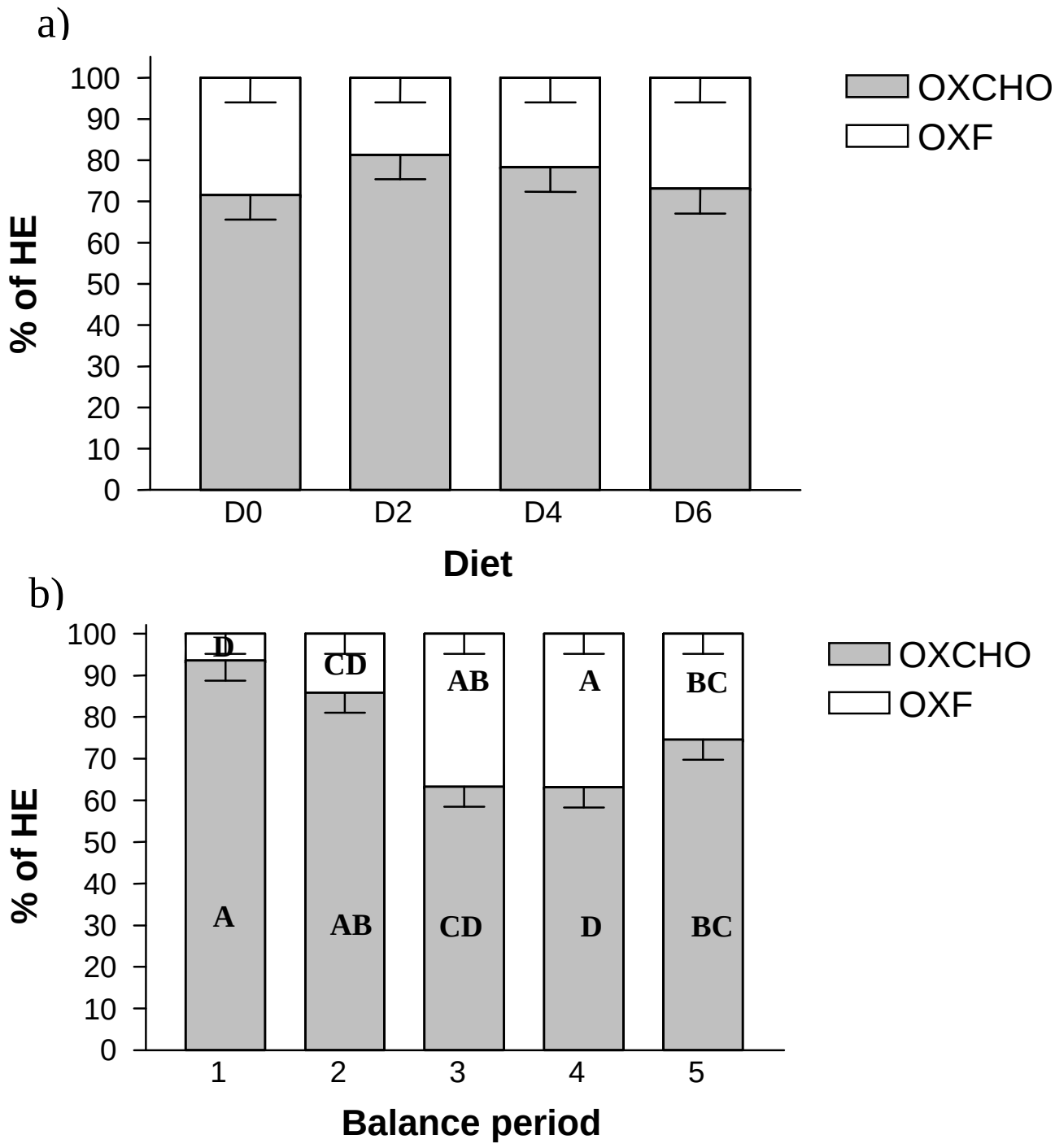

Figure 1. Oxidation of carbohydrate (OXCHO) and fat (OXF) in relation to total heat energy (HE) a) for chickens fed diets with 0% bacterial protein meal (BPM) (D0), 2% BPM (D2), 4% BPM (D4), and 6% BPM (D6). OXF $P = 0.65$ and OXCHO $P = 0.65$ b) for chickens fed diets with a BPM content of 0% to 6% and were measured at 5 different ages, at 3, 10, 17, 23, and 30 days of age. OXF $P < 0.001$ and OXCHO $P < 0.001$.

[A,B,C, D] Values with different letters differ significantly ($P < 0.05$)

Bacterial protein meal in diets for growing pigs – effects on protein and energy metabolism

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Abstract

This experiment investigated the effects of increasing the dietary content of bacterial protein meal (BPM) on the protein and energy metabolism of pigs from weaning until attaining a live weight of 80 kg. A total of four litters with four castrated male pigs in each were used. The litters were divided into two blocks according to time of weaning. One pig from each litter was fed one of the four experimental diets. Soybean meal was replaced with BPM on the basis of digestible protein, and the BPM contents in the four diets were 0% (BP0), 5% (BP5), 10% (BP10) and 15% (BP15), corresponding up to 0%, 17%, 35% and 52% of the digestible nitrogen, respectively. Four balance periods were scheduled, at the start of which the pigs weighed 9.5 kg, 20.7 kg, 45.3 kg and 77.2 kg, respectively. During the same periods, 22-h respiration experiments were performed using indirect calorimetry. Weight gain, feed intake and feed conversion rate as well as the intakes of nitrogen and energy were the same for all diets. The apparent digestibility of nitrogen was significantly lower on diet BP10 than on BP0, whereas the apparent digestibility of energy was similar on all diets. The retention of nitrogen was 1.50, 1.53, 1.33 and 1.46 g N/kg$^{0.75}$ on BP0, BP5, BP10 and BP15, respectively. Although the retention was 0.17 g N/kg$^{0.75}$ lower on BP10 than on BP0, the difference was not significant. Neither metabolizable energy nor heat production were affected by diet. Retention of energy was 620 (BP0), 696 (BP5), 613 (BP10) and 664 kJ/kg$^{0.75}$ (BP15), the differences among diets being non-significant. The N-free respiratory quotient was also similar on all diets. It was concluded that the overall protein and energy metabolism in growing pigs was not affected when up to 50% of dietary N was derived from BPM .

Keywords: Pig, protein metabolism, energy metabolism, bacterial protein meal

Introduction

The global demand for high-quality protein feedstuffs for use in animal nutrition is increasing: fish for the production of fish meal is a limited resource, meat and bone meal and other animal by-products are banned in many countries owing to bovine spongiform encephalitis, and important vegetable protein sources are increasingly genetically modified and thus considered unsuitable for the diets of food animals in many countries. Alternative protein sources thus must continuously be evaluated and, if proved suitable, included in the diets of farm and companion animals. The prerequisites for a protein feedstuff to be considered of high nutritional quality include good palatability, high biological value, harmlessness and being beneficial to product quality. Evaluation studies conducted with fast-growing animals such as pigs and chickens may form a suitable basis for conclusions as to the usefulness of such alternative protein feedstuffs.

Bacterial protein meal (BPM) produced by the continuous aerobic fermentation of natural gas (99% methane) as the energy and carbon source and ammonium as the nitrogen source is one such a new interesting potential protein source (Skrede *et al.*, 1998). The bacterial biomass comprises *Methylococcus capsulatus* (Bath; >90%), *Ralstonia* sp., *Brevibacillus agri* and *Aneurinibacillus* sp.; after fermentation the biomass is spray dried and heat treated to obtain a dry and storage-stable reddish/brown product with a dry matter (DM) content of approximately 96%. The crude protein (CP), fat and ash contents are approximately 70%, 10% and 7%, respectively (Skrede *et al.*, 1998). Nitrogen (N) from the purine and pyrimidine bases in RNA and DNA makes up approximately 12% of the N in BPM, this level being low compared to that of many other single-cell proteins of bacterial origin (Braude *et al.*, 1977; Tiermeyer *et al.*, 1981; Rumsey *et al.*, 1991; Kiessling and Askbrandt, 1993), but considerably higher than that of fish meal (Greife, 1984), wheat, barley, corn or soybeans (Herbel and Montag, 1987; Imafidon and Sosulski, 1990; Lassek and Montag, 1990).

In pig diets, BPM may be used to replace soybean meal (SBM). Compared to that of SBM, the amino acid composition of BPM mainly differs in having a slightly higher content of S-containing amino acids (with a somewhat lower cystine but a higher methionine content). For other essential amino acids, only minor differences between SBM and BPM have been reported (Øverland *et al.*, 2001). The major lipid components of BPM are phospholipids, mainly phosphatidylethanolamine and phosphatidylglycerol with high contents of 16:0 and 16:1 fatty acids (Müller *et al.*, 2004).

Production experiments reported to date suggest that BPM is a promising alternative protein source: dietary BPM providing up to one third of the N intake was found to sustain production performance and animal health in slaughter chickens (Skrede *et al.*, 2003) and blue foxes (Skrede and Ahlstrøm, 2002). When BPM made up approximately 50% of dietary N no adverse effects were reported for growing–finishing pigs (Øverland *et al.*, 2001) or Atlantic salmon (Storebakken *et al.*, 2004); however, when 40–50% of dietary N originated from BPM, reduced performance during the piglet period was noted in some experiments (Øverland *et al.*, 2001 and 2004).

A complete feedstuff evaluation cannot be based solely on performance data, but also needs to consider effects on nitrogen and energy metabolism. A high-quality protein source must sustain high nitrogen retention and not cause elevated heat production (HE). Results regarding BPM are still limited to studies of chicken and mink, which show that retained nitrogen (RN) remained unaffected when BPM made up 20–60% of digestible N in the mink diet (Hellwing *et al.*, 2005a) or 6.5–20% in the chicken diet (Hellwing *et al.*, 2005b). However, the metabolic fate in the organism of the purine and pyrimidine bases in BPM still needs to be elucidated. If these bases are not digested and absorbed, apparent N digestibility will decrease. If they are absorbed but not used in protein metabolism, the excess N is excreted in urine in an energy-expensive process, likely to increase the animals' HE. However, previous findings by Hellwing *et al.* (2005a and b) do not support such a scenario. Data regarding pigs fed yeast RNA in diets sufficient in essential amino acids but low in protein even suggest that RNA might serve as a non-specific N source, as indicated by improved N retention (Roth and Kirchgessner, 1977, 1978).

The present study thus investigates the effects of replacing SBM with increasing dietary levels of BPM in the diets of pigs, from weaning until a weight of 80 kg, on quantitative nitrogen and energy metabolism traits.

Material and methods

Animals and experimental design

In total, 20 recently weaned crossbred castrated male piglets [(Landrace*Yorkshire) * (Hampshire*Duroc)] from five litters were bought from a pig producer. The first three litters (Block A) were delivered one week before the last two litters (Block B). One litter from block A served as spare pigs. One pig from each litter was allocated to each of the four experimental diets. At arrival the piglets in block A weighed 8.1 ± 0.7 kg (mean ± SD) and the piglets in block B 9.8 ± 0.9 kg.

Experimental diets were fed from day one. Nine days after arrival the pigs were placed in metabolism cages and the first 4-day balance period started 12 days after arrival. Another three balance periods were conducted 40, 68 and 96 days after arrival. The pigs weighed 9.5 ± 1.6 kg, 20.7 ± 3.9 kg, 45.3 ± 4.6 kg and 77.2 ± 5.0 kg at the start of the four balance periods, respectively, during which the pigs were also subjected to 22-h respiration experiments by means of indirect calorimetry in an open-air circulation system.

Diets

The BPM (trade name, Bioprotein) was produced and supplied by Norferm AS (Stavanger, Norway). The BPM was pelleted with the inclusion of approximately 1% soy oil after spray-drying. Two batches of feed were produced: the first was used from arrival until the end of balance period 2 (starter diet), and the second was used during the rest of the experiment (growing–finishing diet). The metabolizable energy content of the starter diet was somewhat higher than that of the growing–finishing diet. Wheat was the main ingredient in the starter diet and barley dominated in the growing–finishing diet (Table 1). One of the experimental diets served as the control diet and contained no BPM (BP0), while the remaining diets contained 5% BPM (BP5), 10% BPM (BP10) and 15% BPM (BP15). The BPM replaced soybean meal on a digestible protein basis, comprising 0/0, 17/17, 33/35 and 49/52% of N in the starter and growing–finishing diets, respectively. The diets were formulated to meet or exceed the requirements for essential amino acids and all other nutrients established by the National Research Council (NRC) (1998). The diets were produced by the Center for Feed Technology (Ås, Norway) and pelleted using a 3-mm die on a Münch pellet press (Münch-Edelstahl GmbH, Hilden, Germany). For a more detailed description of the diet formulation, see Øverland *et al.* (2004). The composition and chemical contents of the starter and

growing–finishing diets are given in Table 1, while the contents of amino acids and of purine and pyrimidine bases are presented in Table 2.

Housing and feeding

The pigs were housed individually for the duration of the experiment. For the first 9 days after arrival and between balance periods they were housed in pens with concrete floors covered with wood shavings. The pigs were fed once daily both during and between the balance periods. experimental diets were provided as close as possible to *ad libitum* feeding throughout the experiment. Between balance periods, water was provided *ad libitum* from drinking nipples. During the balance periods, water (twice the weight of the feed) was mixed into the diets. In addition, pigs were given drinking water in a trough. The temperature was kept at 20–22°C throughout the experimental period.

To ensure pig welfare, all pigs were provided with a rubber mat in the front of the metabolism cages during the first and second balance periods, and if considered necessary, also in periods 3 and 4.

Experimental techniques

Pigs were weighed at the start and end of each balance period. The collection of faeces and urine was performed between 8:00 and 12:00 every day. The pigs were fed between 11:30 a.m. and 12:00 a.m. during the balance periods. Urine was collected in 30 ml of 5% sulphuric acid in periods 1 and 2 and in 50 ml of 5% sulphuric acid in periods 3 and 4, except for 2 days in periods 2 and 4 when protein turnover was estimated by means of the ^{15}N-glycine end-point technique (Hellwing *et al*., 2005c).

After collecting the faeces and urine the inside surfaces of the metabolic cage, the mat and collection plate were washed with citric acid. The feed residues, faeces, urine and citric acid rinse were weighed and stored at –18ºC. After each balance period, all the collected material was thawed and mixed to homogeneity except for the citric acid rinse. Samples for chemical analysis were taken and frozen for later use at –18ºC. The citric acid rinse was centrifuged at 3000 × g for 10 min to separate out the solid particles (assumed to be faeces residues) from the fluid citric acid rinse

(assumed mainly to contain N residues derived from urine). The sediment was weighed and freeze-dried. A sample of the supernatant was stored at –18ºC.

The animals were brought to the respiration unit between 9:30 and 10:30. Each respiration experiment lasted 22 h, starting at 11:00 and ending at 9:00 the following morning. The volume of each respiration chamber was 3500 L and the chambers were constructed for animals with live weights of 5–200 kg. For a detailed description of the calibration and measurement procedures, see Chwalibog *et al.* (2004).

Health

Some pigs in block A suffered from diarrhoea and pneumonia in the first week after arrival and were treated with antibiotics. In the first balance period, all pigs were healthy although some had a tendency to loose stools. During the interval between the first and the second periods, one pig in block A and one spare pig died and the post mortem examination showed that death was caused by oedema disease. Another pig with clinical signs of the disease was treated with antibiotics and the remaining pigs were given a prophylactic vaccination. The dead pig from block A was replaced with a spare pig.

Analyses

Samples of diets and freeze-dried faeces were milled and homogenised before analyses. All diets were analysed for dry matter (DM), ash, N, fat, gross energy (GE), amino acids, adenine, guanine, cytosine, thymine and uracil. Feed residues were analysed for DM. Wet faeces were analysed for DM and N and freeze-dried faeces for ash, fat and GE. The supernatant of the citric acid rinse was analysed for N, and the DM content of the sediment was determined by freeze-drying.

DM was determined by evaporation at 105°C to constant weight. Ash was determined by combustion at 525°C. N was determined by the micro-Kjeldahl technique using the Tecator–Kjeltec system 1030 (Tecator AB, Höganäs, Sweden). CP was calculated as N × 6.25. Fat was determined by petroleum ether extraction in a Soxtec system after HCl hydrolysis. GE was determined using an adiabatic bomb calorimeter (IKA Calorimeter system, IKA® Gmbh & Co. KG, Staufen, Germany). The amino acids, except tryptophan, in the diets were determined according to the European Community Directive 98/64/EC (OJ 1998). Tryptophan was analysed according to the Bech-

Andersen procedure (1991). An HPLC method, described in detail by Thode (1999), was used to determine the adenine, guanine, thymine, uracil and cytosine contents of the diets.

Calculations

Carbohydrates (CHO) were calculated by difference. Urinary N (UN) was calculated as the sum of the N in the urine and the citric acid rinse. Faecal N (FN) was calculated as the N content of the faeces plus the N content of the sediment from citric acid rinse. It was assumed that the N content of the sediment was the same as that of the faeces. Energy in urine (UE) was calculated using the factor 53.5 kJ/g × UN (Chwalibog *et al.*, 2004). RN was calculated as ingested nitrogen (IN) minus UN and FN. Metabolizable energy (ME) was calculated as ME, kJ = GE (feed) – energy in faeces (FE) – energy in urine (UE). Heat production (HE) was calculated according to Brouwer (1965) as HE, kJ = 16.18 × O_2, L + 5.02 × CO_2, L – 5.99 × UN, g and retained energy (RE) as ME – HE. The non-protein respiratory quotient (RQ_{np}) was calculated as RQ_{np} = (CO_2, L – [UN, g × 6.25 × 0.774])/(O_2, L – [UN, g × 6.25 × 0.957]).

The HE is the sum of heat produced by the oxidation of protein (OXP), carbohydrate (OXCHO) and fat (OXF). The oxidation of each main nutrient was calculated using the following equations (Chwalibog *et al.*, 1992):

OXP, kJ = UN, g × 6.25 × 18.42

OXCHO, kJ = (–2.968 × O_2, L + 4.174 × CO_2, L – 2.446 × UN, g) × 17.58

OXF, kJ = (1.719 × O_2, L + 1.719 × CO_2, L – 1.963 × UN, g) × 39.76

If RQ_{np} > 1 the calculated OXCHO and OXF do not represent the actual oxidation of nutrients. Therefore, the values were interpreted as the “apparent values”, i.e. AOXCHO and AOXF. In order to calculate OXCHO and OXF for RQ_{np} > 1, it was considered that AOXCHO was overestimated in relation to OXCHO by an amount equal to that of AOXF. OXCHO, kJ was then AXCHO, kJ – AOXF, kJ where AOXF has the reversed sign. OXF was zero. For further description of the theory and the calculations, see Chwalibog *et al.* (1992) and Chwalibog and Thorbek (1995).

Statistical analyses

Statistical analyses of data from the balance and respiration experiments were carried out by means of the MIXED Procedure in SAS® (Littell *et al.*, 1996) using the following model:

$$Y_{ijk} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \gamma_k + \varepsilon_{ijkl}$$

where Y_{ijkl} is the Y_{ijkl}th observation, μ is the general mean, α_i is the fixed effect of diet (BP0, BP5, BP10 and BP15), β_j is the fixed effect of balance period (1 to 4), $\alpha\beta_{ij}$ is the interaction between diet and balance period, γ_k is the fixed effect of block (A and B) and ε_{ijk} is the residual error. Data were analysed as repeated measurements and the heterogeneous autoregressive order 1 (ARH(1)) covariance structure was fitted (Littell *et al.*, 1996). Results are presented as least squares means (LSmeans) and the square root of residuals (RR) is given for each variable. Pair-wise comparisons of LSmeans were made using the PDIFF option, and effects were considered significant if $P < 0.05$. One observation from period 2 was omitted because of an injury not related to the dietary treatment. Another three observations (regarding one pig in period 2 and two pigs in period 3) were omitted from the analysis of the respiration data because of technical problems.

Results

All results regarding intake of nutrients, protein and energy metabolism are presented in relation to metabolic body size ($kg^{0.75}$) in order to facilitate comparisons among balance periods, the exception being intakes of amino acids, which are given in g/day. The block effects observed during the experiment were caused by differences in the weights of the piglets between the two blocks at arrival, the piglets in block B weighing almost 2 kg more than pigs in block A.

Intake of nutrients, digestibility and performance

Effect of diet: Intake of DM was 93 $g/kg^{0.75}$ on diets BP0 and BP10 whereas it was 97 $g/kg^{0.75}$ on BP5 and BP15, the differences between the diets being non-significant. The intakes of CP, carbohydrate and GE reflected the dry matter intake. Because the content of fat in both the starter and growing–finishing diets increased with increasing BPM content (Table 1), the intake of fat increased significantly with increasing dietary BPM (Table 3). The intake of lysine was significantly higher on diets BP0 and BP5 than on BP10 and BP15. The intake of methionine plus cysteine was the same on all diets, but the content of methionine increased whereas the content of cysteine decreased with increasing dietary BPM.

The apparent digestibility of N (ADN) was lowest on diets BP10 and BP15, differing significantly ($P = 0.002$) from the ADN on BP0. The apparent digestibility of fat (ADF) increased significantly ($P < 0.001$) with increasing dietary BPM, whereas the apparent digestibility levels of carbohydrate (ADCHO) and of energy (ADE) were unaffected by diet (Table 3).

The daily gain during the balance periods was lowest on BP10 at 688 g and highest on BP0 at 852 g, but differences between diets were non-significant. The feed conversion rate tended ($P = 0.12$) to become poorer with increasing dietary BPM (Table 3).

Effect of period: Intake of DM, CP, fat, carbohydrate and gross energy increased from period 1 to period 3, but in period 4 the intake of nutrients was intermediate to the levels in periods 1 and 2 (Table 3).

ADN was the same in periods 1 through 3, whereas it was significantly higher in period 4. ADF was highest in period 2 and lowest in period 1. The lower ADF recorded in periods 3 and 4 compared with period 2 was probably caused by the different ingredient composition in the starter diet versus the growing–finishing diet. The ADCHO and ADE were lowest in period 3 at 0.86 and 0.80, respectively. There were small though significant differences in ADCHO among the periods, the highest ADCHO being found in period 1 (Table 3).

The pigs weighed approximately 10, 22, 48 and 79 kg at the midpoint of each balance period. The body weight gain was lowest in the first period and then increased significantly until balance period 3, after which the level remained the same in period 4. The feed conversion rate was most efficient in the first period and then declined to a constant level for the rest of the experiment (Table 3).

Interaction between diet and period: The observed interaction between diet and period for intake of fat, methionine, cystine and tryptophan was mainly caused by that fact that the feed intake relative to metabolic body size was lower in period 4 than in period 3.

Nitrogen metabolism

Effect of diet: Because of the lower DM intake, IN was slightly lower (3.2 g/kg$^{0.75}$) on diets BP0 and BP10 than on BP5 and BP15 (3.4 g/kg$^{0.75}$) but the difference was not significant. The intake of nucleic acid nitrogen (NAN) increased with increasing dietary BPM. Due to the lower IN and ADN, the DN was significantly lower on BP10 than on BP5. The faecal excretion of nitrogen tended to increase (P = 0.12) with increasing dietary BPM. The excretion of N in urine ranged from 0.99 (BP0) to 1.06 g/kg$^{0.75}$ (BP5 and BP15). RN was lowest on BP10 at 1.33 g/kg$^{0.75}$ and highest on BP5 at 1.53 g/kg$^{0.75}$, but the difference was non-significant (P = 0.08). The utilization of digested nitrogen for retention (RN/DN) was close to 60% on BP0 and 55% on BP10, the difference between the diets being non-significant. The partitioning of excreted N between faeces and urine was similar on all diets (Table 4).

Effect of period: Both IN and DN increased significantly until period 3, after which they decreased significantly in period 4. UN excretion was highest in periods 3 and 4, when it differed significantly from the levels in periods 1 and 2. The highest RN values were recorded in periods 2 and 3, when they were significantly higher than in periods 1 and 4. Utilization of DN for retention (RN/DN) was

most efficient in period 2 (64%), after which it declined significantly to below 50% in period 4. This decline was reflected in the partitioning of N excretion between faeces and urine, the latter increasing continuously from period 2 to period 4 (Table 4).

Interaction between diet and period: Animals on diets BP10 and BP15 had a poor utilization of DN for retention in period 1 (not shown), the utilization level being similar to that in period 4. This was likely the main cause of the interaction between diet and period observed for RN/DN. Significant interaction effects for UN and total N excretion could be explained by low UN on diets BP10 and BP15 during period 4.

Energy metabolism

Effect of diet: The intake of ME was highest on diets BP5 and BP15 and lowest on BP0 and BP10, but the differences were non-significant. HE was highest on BP15 but did not differ significantly from the levels on the other diets. RE was not significantly affected by diet. Approximately 35–40% of RE was retained as protein and the rest as fat, and there were no significant differences among the diets. RQ_{np} was unaffected by diet (Table 5). OXP, OXCHO and OXF were the same on all diets (Figure 1a).

Effect of period: ME intake increased from a low level (approximately 1 $MJ/kg^{0.75}$) in period 1 to approximately 1.65 $MJ/kg^{0.75}$ in periods 2 and 3, after which it declined by approximately 0.4 $MJ/kg^{0.75}$ in period 4. This pattern was partly reflected in HE, although the lowest HE values were recorded in period 4. Consequently, RE was low in period 1, increased by almost 0.5 $MJ/kg^{0.75}$ in period 2 and remained at this level in period 3. HE and RE values in the two last periods were probably somewhat affected by the decline in ME intake observed on the days of the respiration experiments (4 g $DM/kg^{0.75}$ in period 3 and 27 g $DM/kg^{0.75}$ in period 4), resulting in RE values in period 4 being lower than expected from the measured body weight gain. RQ_{np} values were also lower than expected (Table 5). The pattern of substrate oxidation reflected both the rate of protein retention, OXP increasing significantly from period 1 to period 4, and the level of ME intake, OXF being highest in period 1 when ME intake was lowest and zero in periods 2 and 3 when ME intake was high (Figure 1b).

Discussion

Intake of nutrients, performance and digestibility

The diets were generally well accepted and the animals performed well even on the diet with highest inclusion level, where BPM supplied approximately 50% of dietary N. The small and non-significant difference in DM intake (approximately 4 g/kg$^{0.75}$) could not be explained by the dietary level of BPM, since the lowest intakes were recorded on the control diet (BP0) and the 10% BPM diet (BP10). However, the differences in DM intake obviously influenced protein and energy intake and some of the other traits measured in the present study. Our results concur with those of Øverland *et al.* (2004): using the same diets as in the present study, they found no differences in feed intake but a small reduction in average daily gain on the diet containing the most BPM.

The amino acid composition differed somewhat between diets, the control diet (BP0) having an almost ideal pattern of essential amino acids (NRC, 1998), whereas increasing BPM inclusion caused gradually lowered lysine content and higher contents of methionine and tryptophan. The lysine contents of all starter diets, declining from 10.7 g/kg feed on BP0 to 8.6 g/kg feed on BP15, were lower than recommended by NRC (1998) for pigs weighing 10–20 kg. Thus the differences in lysine content may have affected the results obtained in the two first study periods. All the growing–finishing diets contained sufficient amounts of lysine, according to the NRC (1998) recommendations.

The intention of this study was to work with intact animals, i.e. animals not equipped with digestive tract cannulas. Therefore, all digestibility data represent total tract digestibility and no determination of digestibility at the terminal ileum was carried out. The ADN of BPM at the terminal ileum and of the total digestive tract of pigs has been estimated to be 0.78 and 0.85, respectively, using BPM as the sole source of protein (Skrede *et al.*, 1998). Regression analysis of our data indicated a lower digestibility, although the equation had a low R^2. However, the ADN of the control diet based on soybean meal was also low, and the effects of replacing SBM with BPM were rather slight. In studies of blue foxes, a BPM-based diet was found to have a significantly lower ileal and numerically lower total tract digestibility than a diet based on SBM (Vhile *et al.*, 2005).

The amino acids in BPM and other bacterial proteins are located both in the cytoplasm and the cell wall, so disruption of the cell wall is necessary to allow digestive enzymes to gain access to the amino acids in the cytoplasm. Despite this fact, Soeder (1977) stated that even a completely indigestible cell wall would only cause a minor decline in N digestibility. The explanation as to why the present study found that BPM consumption resulted in slightly lower ADN levels than those resulting from SBM consumption, may be that *M. capsulatus*, in addition to the cell walls, contains a complex system of poorly digestible internal membranes. Thus, a membrane-reduced extract of autolyzed BPM has been shown to have higher N digestibility than the crude autolyzed BPM does (Schøyen *et al.*, 2005).

The increasing ADF with increasing dietary levels of BPM found in our study was likely due to the increasing fat content of the diets, and hence reduced contributions to faecal fat from endogenous losses (Jørgensen *et al.*, 1992 and 1993). An estimate of the true fat digestibility, assuming an endogenous loss of 4.4 g per kg DM (Jørgensen *et al.* 1993), indicated a higher fat digestibility in BPM than in the fat sources of the control diet. Unlike the results of the mink study (Hellwing *et al.*, 2005a), ADCHO and ADE were not affected by increasing dietary content of BPM. This may be explained by higher levels of dietary carbohydrate – the main energy source in pig diets – and minor contributions from BPM to the carbohydrate fraction. The cell wall carbohydrates in BPM mainly consist of various heptoses, glucose, galactose, *N*-acetylglucosamine, rhamnose and mannose as well as some unusual dideoxy sugars. The digestibility of these compounds is probably low, as has been shown for yeast cell wall glucans (Longe *et al.*, 1981).

Protein metabolism

Our diets were designed to be iso-nitrogenous, and protein metabolism traits were generally found to be independent of dietary BPM. The exception was the low ADN and DN on diet BP10, which was at least partly caused by lower DM intake. Despite less protein being available for retention and the sub-optimal lysine supply, the RN was only slightly and non-significantly lower than the highest value, which was found on diet BP5. This result concurs with our findings in mink, where RN was unaffected by dietary BPM level (Hellwing *et al.*, 2005a). In slaughter chickens, Hellwing *et al.* (2005b) also found similar RN levels for all BPM-containing diets.

The utilization of DN for N retention was 9% higher on diet BP0 than on BP10, but the N derived from nucleic acids increased from 2.5% on BP0 to approximately 10% on BP15 and this fraction cannot be directly used for protein synthesis. The fate of nucleic acid N is important in relation to protein metabolism. Pig experiments have shown that up to 40% of adenine, 15% of guanine and 20% of the pyrimidine bases in the diet were retained in the body (Greife and Molnar, 1984a and b). In addition, some NH_3 released during the decomposition of the purine and pyrimidine bases might be used in the synthesis of non-essential amino acids, as has been shown in the case of yeast RNA in diets adequate in essential amino acids (Roth and Kirchgessner, 1978). The high values for RN on diets BP5 and BP15 indicate that some nucleic acid N may have been used for the synthesis of non-essential amino acids or have been directly deposited in the body.

The retention of protein in different balance periods was in good agreement with results obtained by Whittemore *et al.* (1988), although the highest retention we found was in balance period 3 at an average pig live weight of 48 kg, while Whittemore *et al.* (1988) recorded maximum protein retention at 75 kg live weight. To achieve maximum protein retention the supply of both digestible protein and ME must be sufficient. The criteria for this (DN > 1.9 g/kg$^{0.75}$, ME > 1100 kJ/kg$^{0.75}$; Chwalibog *et al.*, 1996; Tauson *et al.*, 1998) were fulfilled in this study. The pattern of protein retention can be modelled according to a second order function. Data pertaining to pigs of mixed sexes from 2 to 120 kg (Chwalibog *et al.* 1996) or intact boars (Tauson *et al.* 1998) gave peak values of 180 g/day at 98 kg and 227 g/day at 135 kg, respectively. Using a similar approach with the present material gave a peak value of 210 g/day at 62 kg, but the equation had a significant negative intercept, so the result has only indicative value. Considering the genetic progress and effects of sex, our estimated maximum protein retention level seems reasonable, even though it was achieved at a lower than expected live weight.

Energy metabolism

Dietary supply of BPM was not found to affect HE in the present study, which is in agreement with the results of previous studies of mink (Hellwing *et al.*, 2005a) and slaughter chickens (Hellwing *et al.*, 2005b). This means that even assuming 100% digestion of the purine and pyrimidine bases, the energy cost for the excretion of their metabolites is small in relation to other metabolic processes. The ME intake was the same on all diets and hence the RE was unaffected by diet.

Excretion of UN was the same on all diets, resulting in a similar rate of protein oxidation, and the level found here was in agreement with that found by Chwalibog *et al.* (1992) for pigs fed balanced diets. We found that oxidation of carbohydrate was the main metabolic fuel, but in contrast to the results of Chwalibog *et al.* (1992), some fat was found to be oxidized. However, we found no dietary effects on the rates of carbohydrate and fat oxidation.

Until period 3, HE increased linearly in relation to the metabolic body size of the pigs, as also shown by Thorbek (1975). In period 4, however, the lowest HE values were recorded, and RQ_{np} was found to be lower than in periods 2 and 3, which was caused by a lower feed intake during the respiration experiments. During the last period the pigs were observed to spend most of the time sleeping without signs of hunger or discomfort. The feed intake during the complete balance period did not differ between diets, so between -diet comparisons are still valid, whereas comparisons to other balance periods should be made with caution.

In agreement with Chwalibog *et al.* (1992) and with our previous results regarding mink (Hellwing *et al.*, 2005a), oxidation of protein was found to increase with increasing period number, which is in line with the progressively decreasing protein requirement. Fat oxidation was zero during periods 2 and 3, as would be expected of pigs fed at a high level of ME intake with diets containing high carbohydrate levels (Chwalibog *et al.*, 1992, 1998 and 2001). In periods 1 and 4 oxidation of fat occurred; this was probably caused by the lower intake of ME, which is in agreement with Chwalibog *et al.* (2001).

Conclusion

The present data suggest that BPM providing up to 50% of dietary N in the diets of growing pigs can support normal performance as well as normal protein and energy metabolism. With increasing dietary levels of BPM, the amount of amino acid N decreased, but the animals were still able to maintain a high protein retention. This indicated a slightly more efficient utilization of dietary amino acid N, and possibly that some nucleic acid N might have been used for the synthesis of non-essential amino acids or been directly retained in nucleic acids in the body.

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Table 1. Composition and chemical content of diets used in balance and respiration experiments with bacterial protein meal as protein source for growing pigs. Data are expressed in g kg^{-1} and for gross energy in MJ kg^{-1}.

	Starter diet				Growing–finishing diet			
	BP0	BP5	BP10	BP15	BP0	BP5	BP10	BP15
Barley	226	246	250	270	487	518	541	563
Wheat	480	480	500	497	250	250	250	250
Bacterial protein meal (BPM)	0	52	101	153	0	50	100	150
Soybean meal (45% crude protein)	210	140	68	0	224	146	72	0
Soy oil	40	40	40	40	6.0	3.6	5.0	5.0
Limestone	15.8	16.2	16.6	16.9	14.2	14.2	14.2	14.2
Monocalcium phosphate	15.4	14.8	14.4	13.9	11.7	11.7	11.8	11.8
Sodium chloride	4.7	4.6	4.5	4.40	3.8	3.7	3.6	3.5
Iron fumarate	0.33	0.33	0.33	0.33				
Zinc oxide	0.10	0.10	0.10	0.10				
Premix starter diet[†]	1.55	1.55	1.55	1.55				
Premix growing–finishing diet[‡]					1.2	1.2	1.2	1.2
L-lysine HCl (98%)	3.4	2.5	1.8	0.7	0.65	0.64	0.51	0.34
DL-methionine	0.30	0.0	0.0	0.0	0.54	0.32	0.06	0.0
L-threonine	1.0	0.63	0.4	0.0	0.60	0.37	0.09	0.0
L-tryptophan	0.15	0.21	0.3	0.35				
Choline chloride	0.84	0.84	0.84	0.84	0.6	0.6	0.6	0.6
Ascorbic acid	0.60	0.60	0.6	0.60				
Vitamin E	0.04	0.04	0.04	0.04				
Chemical composition								
Dry matter	919	917	918	916	881	886	892	890
Ash	67.4	59.3	59.6	59.0	55.8	54.9	50.5	54.6
Nitrogen	31.7	31.7	32.0	32.9	30.6	31.5	30.3	30.4
Crude protein (N * 6.25)	198	198	200	206	191	197	189	190
Fat	36.9	40.3	52.1	73.5	27	32	40	43
Carbohydrate[§]	617	619	606	578	608	603	612	603
Gross energy	17.5	17.4	17.5	17.8	16.0	16.3	16.7	16.6

[†] Vitamins and trace elements included to provide the following per kg of feed: 140 mg of Zn; 201 mg of Fe; 80 mg of Mn; 20 mg of Cu; 10 mg of I, 0.4 mg of Se; 3 300 μg of vitamin A; 34.4 μg of cholecalciferol; 137.5 mg of d-α-tocopheryl acetate; 6.9 mg of riboflavin; 22.9 mg of d-pantothenic acid; 27.5 μg of cyanocobalamine.

[‡] Vitamins and trace elements included to provide the following amounts per kg of feed: 105 mg of Zn; 75 mg of Fe; 60 mg of Mn; 15 mg of Cu; 7.44 mg of I; 0.3 mg of Se; 2 520 μg of vitamin A, 17.5 μg of cholecalciferol; 115.9 mg of d-α-tocopheryl acetate; 5 mg of riboflavin; 15 mg of d-panthothenic acid; 20 mg of cyanocobalamine.

[§] Calculated by difference.

Table 2. Content of amino acids (g kg^{-1}) and purine and pyrimidine bases (mg kg^{-1} DM) in diets used in balance and respiration experiments with bacterial protein meal as protein source for growing pigs.

	Starter diet				Growing–finishing diet			
	BP0	BP5	BP10	BP15	BP0	BP5	BP10	BP15
Essential amino acids								
Lysine	10.7	10.2	9.8	8.6	9.9	10.0	9.1	8.2
Methionine	2.8	2.9	3.4	3.8	3.0	3.6	3.5	3.7
Threonine	7.0	6.9	7.1	6.8	7.1	7.4	6.8	6.6
Tryptophan	2.5	2.7	2.9	3.2	2.6	2.9	3.0	3.2
Histidine	4.6	4.6	4.5	4.4	4.9	4.8	4.5	4.2
Phenylalanine	8.9	8.7	8.7	8.4	9.1	9.0	8.3	7.7
Leucine	13.2	13.4	13.9	13.9	13.7	14.0	13.4	12.9
Isoleucine	7.7	7.9	8.1	8.1	8.1	8.1	7.8	7.4
Valine	8.8	9.4	10.1	10.6	9.4	10.0	10.0	9.9
Non-essential amino acids								
Arginine	11.3	11.1	11.3	11.1	12.1	12.0	11.2	10.5
Glutamic acid	44.6	43.1	41.9	39.0	41.4	39.1	35.7	32.8
Glycine	7.6	8.0	8.5	8.8	7.7	8.3	8.3	8.1
Serine	9.5	9.0	8.5	7.9	9.4	9.1	7.9	7.1
Proline	13.9	13.9	13.9	13.7	13.1	13.0	12.8	12.0
Alanine	7.3	8.3	9.4	10.3	7.7	9.0	9.5	9.8
Aspartic acid	15.6	14.9	14.2	13.2	16.9	16.2	14.4	12.7
Cystine	3.1	3.0	2.8	2.5	3.2	2.9	2.6	2.4
Tyrosine	6.5	6.6	6.7	6.4	7.5	7.5	7.1	6.5
Purine bases								
Adenine	392	899	1 272	1 905	446	1 027	1 345	1 743
Guanine	347	770	1 148	1 617	370	876	1 155	1 518
Pyrimidine bases								
Cytosine	506	939	1 328	1 794	522	1 083	1 428	1 783
Uracil	722	1 031	1 177	1 541	790	1 267	1 508	1 732
Thymine	139	208	265	347	126	240	252	284
% of N from purine and pyrimidine bases	2.5	5.0	7.1	9.6	2.7	5.6	7.7	9.7

Table 3. Intake of nutrients (g/kg$^{0.75}$/day) and amino acids (g/day), digestibility of nutrients and performance in pigs (weight in kg, daily gain in g/day and feed conversion rate in kg feed/kg gain) fed increasing levels of bacterial protein meal (BPM) from weaning to a weight of approximately 80 kg.

	Diet				Period				RR[†]	*P* values			
	BP0	BP5	BP10	BP15	1	2	3	4		Diet (D)	Period (P)	D*P	Block
Live weight of pigs					10.1	21.7	47.5	79.1					
Number of pigs (n)	15	16	16	16	16	15	16	16					
Intake of nutrients													
Dry matter	93	97	93	97	67^{D}	107^{B}	118^{A}	88^{C}	0.95	0.38	<0.001	0.26	<0.001
Protein (N*6.25)	20	21	20	21	15^{D}	24^{B}	25^{A}	19^{C}	0.87	0.15	<0.001	0.07	<0.001
Fat	3.2^{d}	3.9^{c}	4.7^{b}	6.1^{a}	3.7^{C}	6.0^{B}	4.7^{A}	3.5^{C}	0.15	<0.001	<0.001	<0.001	<0.001
Carbohydrate	63	66	62	64	44^{D}	71^{B}	80^{A}	60^{C}	0.95	0.46	<0.001	0.24	<0.001
Gross energy	1.72	1.81	1.75	1.84	1.28^{D}	2.06^{B}	2.17^{A}	1.62^{C}	0.07	0.27	<0.001	0.38	<0.001
Lysine	16.4^{ab}	17.3^{a}	15.3^{bc}	14.2^{c}	4.3^{D}	12.1^{C}	22.4^{B}	24.5^{A}	0.03	0.003	<0.001	0.07	<0.001
Methionine	4.8^{c}	5.9^{ab}	5.7^{b}	6.4^{a}	1.4^{D}	4.0^{C}	8.3^{B}	9.1^{A}	0.01	<0.001	<0.001	0.001	<0.001
Cysteine	5.2^{a}	5.0^{a}	4.4^{b}	4.2^{b}	1.2^{D}	3.5^{C}	6.7^{B}	7.3^{A}	0.08	<0.001	<0.001	0.004	<0.001
Methionine plus cysteine	10.0	10.9	10.1	10.6	2.6^{D}	7.5^{C}	15.0^{B}	16.4^{A}	0.25	0.17	<0.001	0.07	<0.001
Threonine	11.6	12.5	11.3	11.4	3.0^{D}	8.6^{C}	16.8^{B}	18.4^{A}	0.21	0.10	<0.001	0.08	<0.001
Tryptophan	4.2^{c}	4.9^{b}	4.9^{b}	5.5^{a}	1.2^{D}	3.5^{C}	7.1^{B}	7.7^{A}	0.12	<.001	<0.001	0.01	<0.001
Apparent digestibility													
Nitrogen (ADN)	0.78^{a}	0.77^{ab}	0.75^{c}	0.76^{bc}	0.76^{B}	0.75^{B}	0.75^{B}	0.79^{A}	0.02	0.002	<0.001	0.40	0.49
Fat (ADF)	0.66^{c}	0.74^{b}	0.76^{b}	0.81^{a}	0.71^{C}	0.77^{A}	0.74^{BC}	0.75^{B}	0.001	<0.001	0.001	0.09	0.12
Carbohydrate (ADCHO)	0.88	0.88	0.87	0.87	0.89^{A}	0.88^{B}	0.86^{D}	0.87^{C}	0.01	0.10	<0.001	0.61	0.23
Energy (ADE)	0.82	0.82	0.81	0.81	0.82^{A}	0.82^{A}	0.80^{B}	0.82^{A}	0.01	0.11	<0.001	0.32	0.59
Animal performance													
Live weight	38.1	40.2	40.4	39.7	10.1^{D}	21.7^{C}	47.5^{B}	79.1^{A}	0.43	0.64	<0.001	0.93	<0.001
Daily gain	852	806	688	772	322^{C}	596^{B}	$1\ 150^{A}$	$1\ 050^{A}$	1.00	0.51	<0.001	0.89	0.01
Feed conversion rate	1.8	1.9	1.9	2.1	1.4^{B}	2.0^{A}	2.0^{A}	2.1^{A}	0.25	0.12	<0.001	0.02	0.64

[†] Residual error.

[a,b,c] Values with different superscripts differ significantly, effect of diet ($P < 0.05$).

[A,B,C,D] Values with different superscripts differ significantly, effect of period ($P < 0.05$).

Table 4 Nitrogen metabolism in pigs fed increasing levels of bacterial protein meal (BPM) from weaning to a weight of approximately 80 kg. Ingested nitrogen (IN), ingested nitrogen minus nucleic acid nitrogen (IN–NAN), digested nitrogen (DN), urinary nitrogen (UN), faecal nitrogen (FN), retained nitrogen (RN) and N excretion (g/kg$^{0.75}$/day) and utilization of DN for RN (%).

	Diet				Period				RR[†]	*P* values			
	BP0	BP5	BP10	BP15	1	2	3	4		Diet (D)	Period (P)	D*P	Block
Live weight of pigs					10.1	21.7	47.5	79.1					
Number of pigs (n)	15	16	16	16	16	15	16	16					
IN	3.21	3.40	3.18	3.38	2.33^{D}	3.76^{B}	4.07^{A}	3.03^{C}	0.14	0.15	<0.001	0.07	<0.001
IN–NAN	3.13	3.22	2.95	3.06	2.19^{D}	3.52^{B}	3.80^{A}	2.84^{C}	0.13	0.10	<0.001	0.09	<0.001
DN	2.51^{ab}	2.62^{a}	2.37^{b}	2.55^{ab}	1.78^{D}	2.82^{B}	3.07^{A}	2.39^{C}	0.13	0.04	<0.001	0.23	<0.001
FN	0.73	0.82	0.84	0.86	0.62^{B}	0.98^{A}	1.01^{A}	0.64^{B}	0.002	0.12	<0.001	0.13	0.14
UN	0.99	1.06	1.01	1.06	0.67^{C}	0.97^{B}	1.26^{A}	1.22^{A}	0.09	0.44	<0.001	<0.001	0.12
RN	1.50	1.53	1.33	1.46	1.04^{B}	1.81^{A}	1.80^{A}	1.16^{B}	0.13	0.08	<0.001	0.53	<0.001
RN/DN	59.8	58.0	54.4	56.7	57.8^{B}	64.1^{A}	58.5^{B}	48.5^{C}	0.94	0.15	<0.001	0.001	0.01
N excretion	1.73	1.87	1.86	1.92	1.29^{C}	1.95^{B}	2.27^{A}	1.87^{B}	0.08	0.35	<0.001	<0.001	<0.001
- in faeces, %	43	44	45	45	48^{AB}	50^{A}	44^{B}	35^{C}	0.87	0.90	<0.001	0.09	0.004
- in urine, %	57	56	55	55	52^{BC}	50^{C}	56^{B}	65^{A}	0.87	0.90	<0.001	0.09	0.004

[†] Residual error.

[a,b] Values with different superscripts differ significantly, effect of diet ($P < 0.05$).

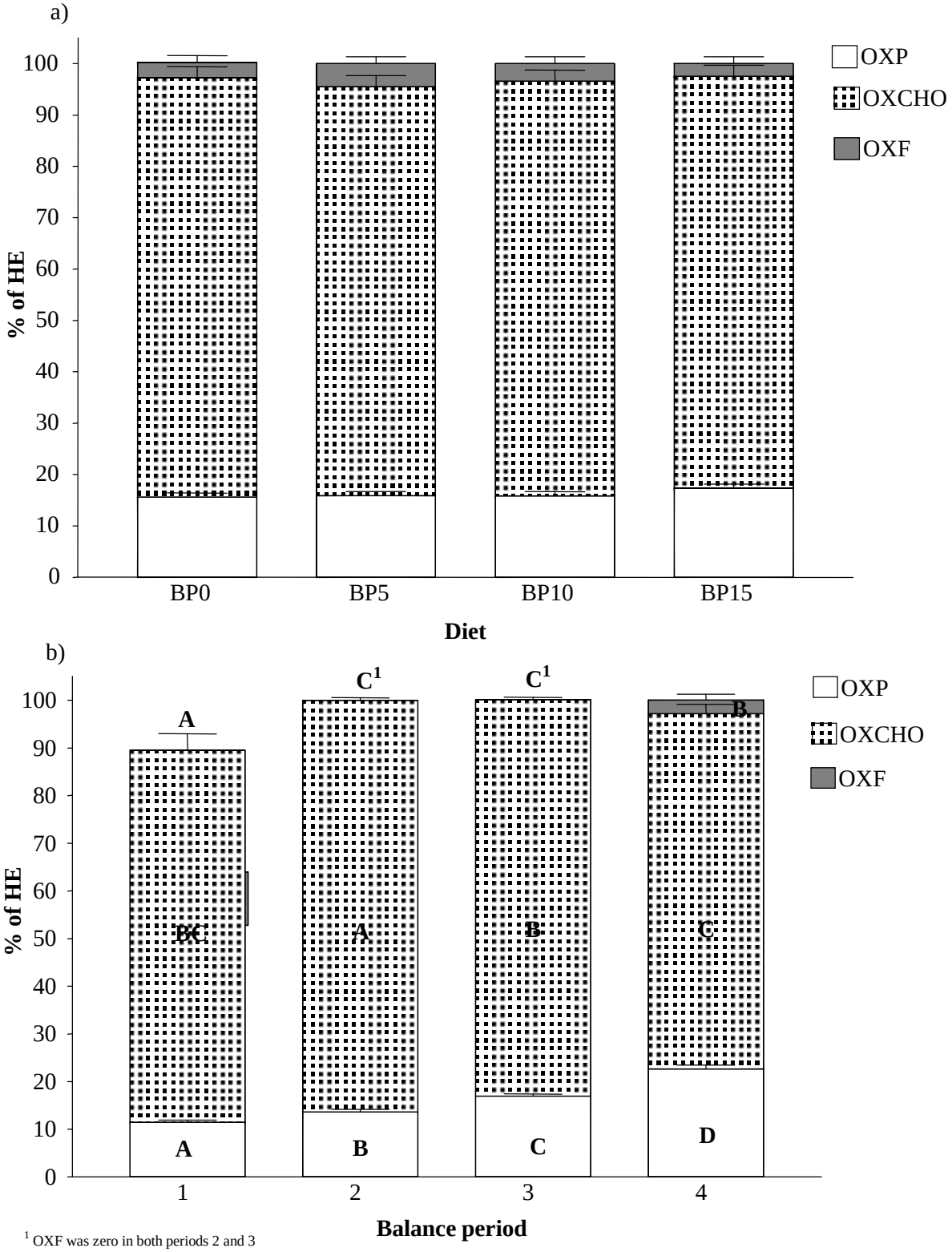
[A,B,C,D] Values with different superscripts differ significantly, effect of period ($P < 0.05$).

Table 5. Energy metabolism in pigs fed increasing levels of bacterial protein meal (BPM) from weaning to a weight of approximately 80 kg. Metabolizable energy (ME), heat production (HE), retained energy (RE), energy retained in protein (RPE), energy retained in fat (RFE) (kJ/kg$^{0.75}$/day) and N-free respiratory quotient (RQ_{np}).

	Diet				Period				RR[†]	*P* values			
	BP0	BP5	BP10	BP15	1	2	3	4		Diet (D)	Period (P)	D*P	Block
Live weight of pigs					10.1	21.7	47.5	79.1					
Number of pigs (n)	14	16	15	15	16	14	14	16					
ME	1 362	1 426	1 360	1 442	1 021^{C}	1 637^{B}	1 677^{B}	1 255^{C}	0.89	0.22	<0.001	0.66	<0.001
HE	741	730	749	777	678^{C}	821^{B}	869^{A}	629^{D}	0.98	0.29	<0.001	0.43	0.001
RE	620	696	613	664	340^{C}	819^{A}	808^{A}	625^{B}	0.85	0.25	<0.001	0.92	0.01
RPE	220	226	195	217	154^{B}	265^{A}	267^{A}	172^{B}	0.97	0.07	<0.001	0.53	<0.001
RFE	400	470	419	446	185^{C}	554^{A}	543^{A}	454^{B}	0.90	0.33	<0.001	0.85	0.51
RQ_{NP}	1.07	1.06	1.04	1.05	0.97^{C}	1.08^{B}	1.12^{A}	1.05^{B}	0.04	0.16	<0.001	0.50	0.55

[†] Residual error.

^{A,B,C,D} Values with different superscripts differ significantly, effect of period ($P < 0.05$).



[1] OXF was zero in both periods 2 and 3

Figure 1. Oxidation of protein (OXP), fat (OXF) and carbohydrates (OXCHO) in percent of total heat production (HE) a) pigs fed diets with 0% bacterial protein meal (BPM) (BP0), 5% BPM (BP5), 10% BPM (BP10) and 15% BPM (BP15). *P* values, effect of diet: OXP = 0.47, OXF = 0.72 and OXCHO = 0.92. b) for pigs fed diets with a BPM content from 0% to 15% and measured in four balance periods at an average live weight of 10.1 kg, 21.7 kg, 47.5 kg, and 79.1 kg. *P* values, effect of period: OXP < 0.001, OXF < 0.001 and OXCHO < 0.001.

[A,B,C, D] Values with different capital letters differ significantly ($P < 0.05$)

Bacterial protein meal in diets for pigs and minks – protein turnover and urinary excretion of purine base derivatives

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The effect of increasing the dietary content of bacterial protein meal (BPM) on protein turnover, and on nucleic acid and creatinine metabolism in growing minks and pigs was investigated in two experiments. In each experiment, sixteen animals were allocated to four experimental diets. The diets containing no BPM served as controls, i.e. (Mink(M)1, Pig(P)1); the experimental diets contained increasing levels of BPM to replace fish meal (minks) or soybean meal (pigs), so that up to 17% (P2), 20% (M2), 35%(P3), 40% (M3), 52% (P4), and 60% (M4) of digestible N was BPM derived. Protein turnover was measured by means of end-product methods using [^{15}N]glycine as tracer and urinary nitrogen as end-product. In minks, protein flux, synthesis, and breakdown increased significantly with increasing dietary BPM. In pigs, diet had no observed effect on protein turnover. The intake of nucleic acid nitrogen (NAN) increased from 0.15 g/kg$^{0.75}$ on M1 to 0.26 g/kg$^{0.75}$ on M3 and M4 in the mink experiment, and from 0.08 g/kg$^{0.75}$ on P1 to 0.33 g/kg$^{0.75}$ on P4 in the pig experiment. Increased NAN intake led, in both experiments, to increased allantoin excretion. Analysis of species effects showed that minks excreted 1.72 g/kg$^{0.75}$ of allantoin, significantly more than the 0.95 g/kg$^{0.75}$ excreted by pigs. In minks, approximately 96% of the excreted purine base derivatives consisted of allantoin, whereas in pigs approximately 93% did. Thus, increasing the dietary content of BPM increased protein turnover in minks but not in pigs, and allantoin excretion increased with increasing dietary BPM.

Introduction

Bacterial protein meal (BPM) is a protein source that offers a potential alternative to fish meal, meat and bone meal, soybean meal, and other vegetable protein sources in animal nutrition. In experiments with minks, chickens, and pigs it has been shown that up to 40% (minks), 20% (chickens), and 50% (pigs) of dietary N can be supplied by BPM without affecting nitrogen retention, heat production, and energy retention (Hellwing *et al.* 2005*a,b,c*). Similarly, production experiments with chickens (Skrede *et al*. 2003) and pigs (Øverland *et al.* 2001, 2004) have shown that up to one third (chickens) and 50% (pigs) of the N can be derived from BPM without negative effects on animal performance and production traits.

BPM is produced by the continuous fermentation of natural gas (99% methane) as the carbon and energy source and ammonium as the nitrogen source by the bacteria *Methylococcus capsulatus* (Bath) (>90%), *Ralstonia* sp., *Brevibacillus agri*, and *Aneurinibacillus* sp. (Skrede *et al*. 1998). The final dried product is a reddish/brown meal comprising approximately 96% dry matter, 70% crude protein, 10% lipids, and 7% ash. Approximately 12% of N in BPM is derived from nucleic acid N in RNA and DNA, although the RNA and DNA contents are relatively low compared to those of other bacterial protein sources (Braude *et al*. 1977; Tiemeyer *et al*. 1981; Rumsey *et al.* 1991; Kiessling & Askbrandt, 1993). Compared to fish meal, the content of RNA and DNA is high in BPM (Greife, 1984a). The amino acid pattern of BPM is similar to that of fish meal, except for somewhat lower lysine and higher tryptophan contents. Compared to soybean meal (SBM), in BPM the methionine content is slightly higher and cysteine is slightly lower, resulting in a higher total combined content of methionine and cysteine in BPM than in SBM.

Dietary RNA and DNA are decomposed into nucleic acids in the intestinal lumen, and further decomposed into nucleosides and free purine and pyrimidine bases by nucleoside phosphate enzymes in the mucosa (Privat de Garilhe, 1967). The nucleosides and the free purine and pyrimidine bases can either be used directly or further decomposed. Some of the intermediate products of the decomposition process can be salvaged to form new nucleotides. The utilisation of dietary purine and pyrimidine bases seems to be quite complicated, and it depends on the type of base and on the nutritional state and species of the animal (Savaiano & Clifford, 1978; Greife & Molnar, 1978*a,b*; Ho *et al*. 1979; Yokozawa *et al.* 1982, 1983; Greife, 1984b; Greife & Molnar,

1983, 1984*a,b*; Brulé *et al.* 1988; Berthold *et al.* 1995). From human studies of gout it is known that high protein levels and a high intake of purine bases increase the production and excretion of uric acid. However, the uricogenic effect of the different bases depended on type of base and whether it was given in the free base, nucleoside, or nucleotide form (Brulé *et al.* 1988). Pigs fed either yeast RNA or bacterial protein had an increased plasma concentration of allantoin and an increased excretion of purine derivatives in urine (D'Mello *et al.* 1976; Braude *et al.* 1977; Roth & Kirchgessner, 1977*a,b*, 1978; Greife *et al.* 1984).

It is not known whether the increased load of purine bases as such affects protein turnover, but at a given dietary N level, the intake of amino acids from BPM-containing diets will decrease. In pigs protein quality has been shown to influence protein turnover (Saggau *et al.* 2000), while in cats, medium levels of protein gave a lower flux than a protein-rich diet did (Russell *et al.* 2003). In minks, the decarboxylation of 1-^{13}C-leucine, as measured by breath testing, was not influenced by protein source (Tauson *et al.* 2000), but by protein supply (Tauson *et al.* 2001*a*).

In both minks and pigs, increased intake of RNA and DNA may lead to changes in the excretion of purine derivatives in urine, though the response can be expected to differ between species due to differences in gastrointestinal tract anatomy, nutrient requirements, and nitrogen metabolism regulation. For example, the mink is a strict carnivore with a very short gastrointestinal tract and a high protein requirement, whereas the pig is an omnivore selected for high body weight gain and the efficient utilisation of nutrients.

The present study investigates the impact of increasing the dietary supply of BPM on the whole-body protein turnover and on the excretion pattern of purine base derivatives in growing pigs and minks, and seeks to reveal possible species differences.

Material and methods

Two nitrogen balance and respiration experiments, one with minks and the other pigs, were carried out. During the balance periods, protein metabolism was studied by means of end-product methods using [^{15}N]glycine as the tracer (minks and pigs) and breath testing (minks). The collected urine was also analysed for purine base derivatives. These experiments are described in detail and the results of the nitrogen balance and energy metabolism studies are reported in Hellwing *et al.* (2005*a*,*c*). The present paper presents data regarding protein turnover, purine base derivative excretion patterns, and between-species comparative aspects.

Animals and experimental design

Minks. Sixteen male mink kits of the standard brown colour type were divided into two blocks according to time of birth, and allocated to four treatment groups as described by Hellwing *et al.* (2005*a*). The animals were measured in balance and respiration experiments (indirect calorimetry in an open-air circulation unit) in their 10th (period 1), 15th (period 2), 18th (period 3), 24th (period 4), and 29th (period 5) week of life. During the balance periods, the animals were housed in metabolic cages in the laboratory under natural light conditions (55°N 12°E) and at room temperature. The metabolic cages were constructed according to the principles presented by Jørgensen and Glem-Hansen (1973), and had dimensions of 66 cm × 31 cm × 47 cm. In the interval between balance periods the animals were kept under conventional farm conditions and fed a conventional mink diet.

Pigs. Sixteen castrated piglets [(Landrace*Yorkshire) * (Hampshire*Duroc)] from four litters were divided into two blocks according to time of weaning. One pig from each litter was allocated to each of the four treatment groups. The pigs weighed 10.1 ± 1.8 kg (period 1), 21.9 ± 4.0 kg (period 2), 47.6 ± 4.7 kg (period 3), and 79.3 ± 5.0 kg (period 4) on the first day of each of four balance periods. During the balance periods, the animals were housed in metabolic cages in the laboratory at an ambient temperature of 20–22°C. Between the balance periods, the pigs were kept in individual pens in the stable (see Hellwing *et al.* 2005*c*).

Diets and feeding routines

BPM. The BPM used in the mink diets was derived from an experimentally produced batch, whereas that used in the pig diets was produced commercially and pelleted before being delivered by Norferm AS (Stavanger, Norway).

Minks. One experimental diet contained no BPM and served as the control diet (M1); in the other diets BPM replaced high-quality fish meal, so that BPM supplied 20% (M2), 40% (M3), and 60% (M4) of the digestible N. The formulation and chemical composition of the diets are given in Table 1. The animals were fed as closely as possible to *ad libitum* feeding, and feed was offered once a day. Water was freely available at all times. For further details, see Hellwing *et al*. (2005*a*).

Pigs. The pigs were fed once daily as closely as possible to *ad libitum* feeding, from weaning until the end of the experiment. Two sets of diets were used, one starter and one growing–finishing. The pigs were switched to the growing–finishing diets directly after the end of balance period 2. The ingredients in both sets of diets were the same (for details see Øverland *et al*. 2004). One of the four diets served as the control diet (P1), in which approximately 50% of the N was supplied by soybean meal (SBM). In the other diets, SBM was replaced with BPM so that approximately 17/17% (P2), 33/35% (P3), and 49/52% (P4) of the N was derived from BPM in the starter/growing–finishing diets, respectively. Diet formulation and chemical composition are given in Table 2. For further details see Hellwing *et al*. (2005*c*).

Collection procedures, data recording, and balance experiments

Both minks and pigs were weighed at the start and end of each balance period. Feed residues, faeces and urine were collected quantitatively daily, weighed, and then frozen at –18°C. After each collection period, the metabolism cages, collection screens, and funnels were rinsed with 5% citric acid. Urine was collected in 5% sulphuric acid, the exception being days when [^{15}N]glycine was used to study protein turnover (see below). At the end of each balance period feed residues, faeces, and citric acid rinse were thawed and mixed to homogeneity. Samples for dry matter and nitrogen analyses were taken. Urine samples were also thawed and mixed to homogeneity (not, however, the samples taken for ^{15}N analyses). Urine collected during the balance periods was sampled for N and for purine derivative analyses, and samples were stored at –18°C pending analysis. Urine collected

during the protein turnover studies was divided into two samples, one for ^{15}N and one for N analyses.

^{15}N-glycine endpoint technique

Protein turnover was studied by means of an end-product method using [^{15}N]glycine as the tracer; protein turnover was studied in minks in all periods, but in pigs only in periods 2 and 4. Before administration of the [^{15}N]glycine urine was sampled from all animals in order to establish a baseline value for ^{15}N. All samples were weighed, so that the content of excreted N could be included in the N balance calculations.

Minks. 1-^{13}C (99.0 atom% ^{13}C), ^{15}N (99.9 atom% 15N) glycine was used (Campro Scientific, Veenendaal, Netherlands) as the substrate, because the experiment was combined with a breath test (see below) conducted on the same day as the animals were measured in the respiration experiment. Immediately before the start of the respiration experiment, the animals were given an intraperitoneal injection of 1 ml/kg body weight of a solution containing 5 mg of [^{15}N][1-^{13}C] glycine dissolved in 1 ml of isotonic saline. Owing to the construction of the chambers, urine could not be sampled during the respiration experiments. Therefore, a pilot study of adult male minks was conducted using the same diets, in order to establish the excretion curve for the label. Urine was collected 3, 6, 9, 12, 15, 24, 36, and 48 h after the administration of [^{15}N] glycine. The results suggested that the 24-h cumulative excretion of ^{15}N could be used in the calculation of protein flux, breakdown, and synthesis, and that the label excreted later could be assumed to be recycled (Tauson and Bujko, unpublished data).

Pigs. The dose (5 mg/kg body weight of [^{15}N]glycine, 99.9 atom%; Campro Scientific, Veenendaal, Netherlands) was administered in 150 g of feed suspended in 100–150 g of water. The pigs were given 15 min to consume the feed. The urine excreted after 3, 6, 9, 12, 15, 24, 36, and 48 h was collected, weighed, and then stored at –18°C for later analyses of N and ^{15}N.

Breath testing

Breath testing was performed with minks in periods 1, 3, 4, and 5 on the days of the respiration experiments, because it was then possible simultaneously to measure CO_2 production and the C^{13}/C^{12} ratio. Substrate and dose were as described above (end-product methods with [^{15}N]). The animals were brought to the respiration chambers at least half an hour before the respiration experiment started, inserted into the chambers, and the chambers were closed. Ten minutes before the start of the respiration experiment, the baseline $^{13}C/^{12}C$ ratio was measured. Immediately before the start of the respiration experiment, the chambers were quickly opened and the dose administered by intraperitoneal injection. The $^{13}C/^{12}C$ ratio in breath air was then measured every 10 min for the next 4 h.

Blood samples

In the pig experiment, fasting blood samples were taken from the jugular vein once per period; two samples were collected, one in a heparin-coated and another in an EDTA-coated vacutainer tube. The samples were chilled on ice, and the plasma was separated by centrifugation for 20 min at 3000 rpm at 4°C. The plasma was frozen at –18°C pending analysis.

Analyses

The DM in feed, feed residues, and faeces was determined by evaporation at 105°C to constant weight. The N content was determined in faeces, urine, citric acid rinse, and feed by means of the micro-Kjeldahl technique using the Tecator-Kjeltec system 1030 (Tecator AB, Höganäs, Sweden). The ^{15}N content of urine was measured by means of emission spectroscopy using an NOI 7 spectrometer (Fischer Analysen Instrumente GMBH (FAN), Leipzig, Germany). HPLC was used to determine the contents of adenine, guanine, thymine, uracil, and cytosine in the diets, and the contents of creatinine and the purine derivatives hypoxanthine, xanthine, uric acid, and allantoin in urine (minks and pigs) and plasma (only pigs; allantoin not analysed). For a detailed description of the procedures, see Thode (1999). The $^{13}C/^{12}C$ ratio in air from the mink respiration chambers was analysed using an IRIS infrared analysing system (Wagner Analysentechnik, Bremen, Germany) and the results were given in delta values, $\delta\ ^{13}C$.

Calculations

Retained nitrogen (RN) was calculated as nitrogen intake, minus the nitrogen excreted in the faeces and urine and contained in the citric acid rinse. Protein turnover was calculated according to the single pool model proposed by Sprinson and Rittenberg (1949). When the free amino acid pool is constant, the turnover rate (termed flux, Q) is given as $Q = S + E = B + I$, where S is synthesis of N, E is excretion of N, B is breakdown of N, and I is intake of N (digested nitrogen). The flux, Q, can be estimated as $Q = E * d/e$, where E is the rate of N excretion, d is the dose given, and e is the cumulative excretion of ^{15}N in urine over 24 h (mink) or 48 h (pigs). The net protein synthesis was calculated as the difference between synthesis and breakdown.

The $\delta\ ^{13}C$ values were converted to ^{13}C enrichments E (in atom % excess) for the calculation of ^{13}C recoveries (Klein, 1991). The recovery (f) as a percentage of the ^{13}C dose (D) after administration of [1-^{13}C][^{15}N]glycine was calculated from the cumulative products of ^{13}C enrichment, $E((t_{i+1} + t_i)/2)$ in breath CO_2 (in atom% of excess) and the CO_2 amount of $nCO_2(t_{i+1} - t_i)$ produced in consecutive time periods $(t_{i+1} - t_i)$:

$$f(\%\text{ of }^{13}\text{C dose}) = [\sum_{i=1}^{N} E((t_{i+1} + t_i)/2) \times nCO_2(t_{i+1} - t_i)]/D$$

The relative growth rates of mink and pigs were calculated according to Brody (1945):

$$k = (\ln W_2 - \ln W_1)/A_2 - A_1)$$

where W_1 and W_2 are animal live weights in g, and A_1 and A_2 are animal ages in days at the beginnings and ends of the balance periods, respectively.

Statistical analyses

Four different models were used to analyse the data. All statistical analyses were performed using the Statistical Analysis Systems (SAS) statistical software package, version 8.0 (SAS Institute Inc., Cary, NC, USA). The results of all analyses are presented as least square means (LSmeans).

Analyses performed using the MIXED procedure are presented with square root of residuals (RR) while analyses performed using general linear models (GLM) are presented with root mean square error (RMSE) as measures of variance. Pairwise comparisons were made using the PDIFF option, and effects were considered significant if $P < 0.05$.

Minks. All data except for those derived from the breath test were analysed using the GLM procedure in SAS (SAS Institute Inc., 1990) according to the following model:

$$Y_{ijkl} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \gamma_k + \varepsilon_{ijkl} \quad \text{(Model A)}$$

Pigs. Data were analysed using the MIXED procedure in SAS as repeated measurements, and the heterogeneous autoregressive order 1 (ARH1) covariance structure was fitted (Littell *et al.* 1996). The following model was used:

$$Y_{ijk} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \gamma_k + \varepsilon_{ijkl} \quad \text{(Model B)}$$

Breath testing, minks. Statistical analyses of the breath testing data were carried out using the MIXED procedure in SAS as repeated measurements, and the autoregressive order 1 (AR1) covariance structure was fitted (Littell *et al.* 1996). The following model was used:

$$Y_{ijklm} = \mu + \alpha_i + \beta_j + \delta_k + (\alpha\beta)_{ij} + (\alpha\delta)_{ik} + (\beta\delta)_{jk} + (\alpha\beta\delta)_{ijk} + \gamma_l + \varepsilon_{ijklm} \quad \text{(Model C)}$$

Analyses of diet and species effects. The dataset for this analysis comprised all data from the mink and pig experiments, except values omitted because of technical problems. Diets containing similar contents of N derived from BPM were paired as follows: D1 contained data from the two control groups M1 and P1; D2 contained data from M2 and P2, etc. Statistical analyses of these data were performed using the GLM procedure in SAS (SAS Institute Inc., 1990) with the following model:

$$Y_{ijk} = \mu + \alpha_i + \kappa_j + (\alpha\kappa)_{ij} + \varepsilon_{ijk} \quad \text{(Model D)}$$

In all models Y denotes the response variable analysed,

μ the general mean,

α the fixed effect of diet,

β the fixed effect of balance period,

δ the fixed effect of time,

κ the fixed effect of species,

$\alpha\beta$ the interaction between diet and balance period,

$\alpha\delta$ the interaction between diet and time,

$\beta\delta$ the interaction between balance and time,

$\alpha\kappa$ the interaction between diet and species,

$\alpha\beta\delta$ the three way interaction effect of diet, balance and time,

γ the effect of block, and

ε the residual error.

Results

The results are presented in relation to metabolic body size ($kg^{0.75}$), in order to facilitate comparisons among periods and species. Significant block effects occurred in both the mink and pig experiments, caused by differences in the weight of the animals in the different blocks. The data for the nitrogen balance and the purine base metabolism are averages of four days of measurements, whereas the data regarding protein turnover cover one day in the mink and an average of two days in the pig study.

Protein metabolism and turnover

Minks. Intake of nitrogen (IN) was lower on M4 than on the other diets, but the differences were not significant (P=0.07). The excretion of faecal nitrogen (FN) increased while that of urinary nitrogen (UN) decreased with increasing content of dietary BPM (Table 3). During the ^{15}N-glycine endpoint study, both the intake (I) and excretion of nitrogen (E) were significantly lower on M4 than on the other diets. Both protein synthesis and breakdown increased with increasing dietary BPM. The protein flux rate appeared to form two groups, with diets M1 and M2 in one and M3 and M4 in the other, but the difference between the two groups was not significant (P=0.08). The net protein synthesis was 5.2 g protein on M1 and 1.7 g lower on M4, but the difference was non-significant (P=0.08).

The protein flux rate was highest during the first three periods when the growth rate of the mink kits was the highest. The net protein synthesis was highest in the first period and decreased significantly with age. In period 5, net protein synthesis was 0.1 g/day, indicating that the animals had reached mature body size (Table 3).

Pigs. Intake of nitrogen was similar on all diets. Excretion of FN increased with dietary content of BPM, but not significantly (P=0.12). Excretion of UN did not differ significantly between diets. Nitrogen retention was 0.17 g lower on P3 than on P1, but the difference was non-significant (P=0.08). Protein synthesis, breakdown, and flux rate were highest on P1 and lowest on P4, the differences being non-significant; however, the rates did not decrease linearly with

increasing dietary BPM content, because the synthesis, breakdown, and flux were higher on P3 than on P2 (Table 4).

IN and excretion of FN and UN were highest in period 3. Though nitrogen retention was highest in period 2, it did not differ significantly from that of period 3. The protein turnover was only measured in balance periods 2 and 4: nitrogen intake (I) was the same in both periods, whereas nitrogen excretion (E) was significantly higher in period 2. Protein synthesis was significantly higher in period 2 than in period 4, while protein breakdown and flux were the same in both periods. Net protein synthesis was higher in period 2 than in period 4 (Table 4).

Breath testing

Neither the oxidation rate (data not presented) nor the cumulative recovery of label were affected by diet. The cumulative excretion of ^{13}C after 240 min was 16.9% (M1), 17.2% (M2), 15.9% (M3), and 15.2% (M4) of the injected label. Balance period had a significant effect on the cumulative excretion, and after 240 min, 15.8% (period 1), 19.2% (period 3), 17.9% (period 4), and 12.3% (period 5) of the ^{13}C was recovered. The recovery was significantly lower in period 5 than in the other periods (Fig. 1).

Nucleic acid and creatinine metabolism

Minks. The intakes of adenine, cytosine, and uracil increased and thymine decreased significantly with increasing dietary content of BPM. Despite a higher dietary content of guanine, on M4, guanine intake was significantly lower than on M3, caused by the lower feed intake (Tables 1 and 5). The total intake of nucleic acid nitrogen (NAN) was 0.15, 0.20, 0.26, and 0.26 g/kg$^{0.75}$ and made up 5.8% (M1), 7.7% (M2), 9.9% (M3), and 11.2% (M4) of the dietary N, respectively. The 24-h urinary creatinine excretion decreased and while that of allantoin increased with increasing dietary content of BPM, whereas uric acid excretion was not affected by diet. The urinary concentration of creatinine decreased whereas that of allantoin increased significantly with increasing dietary BPM content (Fig. 2). On all diets, more purine base derivatives were excreted than were obtained from the diet. Both the ratio between nitrogen derived from creatinine and from purine base derivatives and the ratios between the different purine base derivatives were affected by

diet. The excretion of allantoin increased and that of uric acid decreased in relation to the total purine base derivative excretion when the dietary content of BPM increased (Table 5).

Intake of feed, purine and pyrimidine bases, and NAN remained the same in the first three periods, but then decreased significantly during the last two periods. The 24-h excretion of creatinine, allantoin, and uric acid was highest in period 3 (Table 5).

The interactions between diet and period for the intakes of adenine, cytosine, and uracil were caused by the lower feed intake on M4. In some periods, the intake of these bases was higher on M3 than on M4. The interaction effects on total excretion of purine base derivatives and other N-containing products were caused by a distinctly different excretion pattern in period 1 than in the other periods.

Pigs. Intakes of adenine, guanine, cytosine, uracil, thymine, and NAN increased with increasing dietary content of BPM (Table 6). The 24-h urinary excretion of creatinine was not affected by diet, but allantoin excretion was significantly higher on P4 than on the other diets. The 24-h urinary excretion of uric acid and xanthine increased with increasing dietary content of BPM. The urinary concentration of allantoin increased with increasing dietary content of BPM, but the increase was not significant (P=0.07) (Fig. 2). The ratios between excretion of purine base derivatives and intake of purine bases were 1.26, 0.66, 0.48, and 0.38 on diets P1, P2, P3, and P4, respectively, and the observed decrease with increasing dietary BPM content was significant. With increasing dietary content of BPM, a decreasing fraction of the total excreted purine base derivatives consisted of allantoin, whereas the absolute contents of uric acid and allantoin increased. The plasma concentrations of creatinine, uric acid, xanthine, and hypoxanthine were not affected by diet (Table 6).

The intake of NAN was lowest in period 1 and highest in period 3. The 24-h urinary excretion of allantoin was lowest in period 1 and highest in period 3. Creatinine excretion increased significantly with age; similarly, the plasma concentrations of creatinine, uric acid, and xanthine increased significantly with age (Table 6).

Species effects

Effect of diet. When data were analysed for the two species together, diet effects on the intake and excretion of N and protein turnover traits as found for minks in the separate analyses were alleviated. The apparent digestibility of N decreased with increasing dietary level of BPM (as in the separate analyses of the two species), and this was hence the only N metabolism trait that was significantly affected by diet. The intake of NAN increased significantly with increasing dietary content of BPM. The excretion patterns for creatinine and the purine base derivatives were more clearly pronounced according to this analysis, the only trait not significantly affected by diet being excretion of uric acid. Creatinine excretion showed a clear decrease, whereas allantoin excretion increased steadily with increasing dietary content of BPM (Table 7).

Effect of species. The analysis of effect of species on the investigated traits revealed interesting and clear differences between species. The intake of DM in pigs was approximately double that in minks, resulting in significantly higher IN and DN despite the lower crude protein contents of the pig diets. Similarly, FN and RN were higher, whereas UN was significantly lower, in pigs than in minks. The protein turnover rates were also significantly higher in pigs than in minks. Excretion of allantoin and uric acid was significantly higher in the minks than in the pigs, whereas the opposite was the case for the excretion of creatinine, xanthine, and hypoxanthine. In the minks, allantoin and uric acid made up a relatively greater proportion of the total excretion of purine derivatives than was the case in pigs.

Discussion

The data from the present investigation demonstrate interesting similarities and differences between the investigated species: protein turnover in mink, but not in pigs, was affected by dietary content of BPM, whereas the purine base metabolism was influenced in both species. When comparing the data, however, it must be born in mind that the animals were at different stages of physiological maturity when the experiments were conducted: the first period was conducted shortly after the weaning of both pigs and mink, but during the last period the pigs were still far from their mature body weight, whereas the minks had almost reached mature body size and completed their first fur moulting–priming cycle.

Nitrogen metabolism

In both species, RN was independent of the dietary BPM content. This appeared both when analysing data from each species separately (Hellwing *et al*. 2005*a,c*) and in the comparative analysis. Surprisingly, at first sight IN was higher for the pigs than for the minks, despite the high protein requirement of the minks. However, this can be explained by the higher relative growth rate and higher feed intake of the pigs: although the mink diets had a higher crude protein content, it was not high enough to compensate for the effect of the pigs' higher dry matter intake. The minks excreted more UN than the pigs did, and therefore the utilisation of DN for RN was considerably higher in the pigs than in the minks. To some extent these differences between species reflect differences between the metabolisms of the strict carnivore and the omnivore: strict carnivores, such as the minks in this experiment, are often fed above their minimum protein requirement, and hence use a greater part of the digested amino acids as an energy source than, for example, pigs do. This was demonstrated by the finding that protein accounted for a relatively greater proportion of the total heat production in the minks than in the pigs (Hellwing *et al*. 2005*a,c*).

Protein turnover

The calculated protein flux in minks was, in the present study, based on 24-h excretion data. In the cat (Russell *et al*. 2003) and in the pig (Saggau *et al*. 2000; Krawielitzki *et al*. 1989), 48-h data have previously been used. The choice of cut-off time for ^{15}N excretion is important for the estimation of

the flux. If too short a cut-off time is chosen, the label will be incompletely excreted and the synthesis of protein overestimated, whereas if too long a cut-off time is chosen, some of the label may be recycled and the synthesis of protein underestimated (Waterlow *et al.* 1978). The cut-off time of 24 h for the minks was chosen after a pilot study, in which the cumulative excretion of label was measured for 48 h. From the excretion curve, Tauson and Bujko (unpublished data) concluded that most of the label not used in protein synthesis was excreted after 24 h, and that label excreted later was likely to be recycled. The increase in both synthesis and breakdown for minks with increasing dietary BPM seems, at first sight, contrary to the findings of Reeds *et al*. (1980, 1981), Fuller *et al*. (1987), and Russell *et al*. (2003), who have shown in the pig and cat that a decrease in dietary nitrogen content decreases the protein synthesis. However, an increase in the supply of essential amino acids at a stable level of nitrogen intake increases the protein synthesis and breakdown in pigs (Gotterbarm *et al*. 1998). The amino acid composition of our mink diets was not analysed, but from data by Skrede *et al*. (1998) it can be assumed that dietary methionine, cystine, tryptophan, and threonine increased with increasing dietary content of BPM. This may provide an explanation for the increasing synthesis and breakdown rate, even though the total content of amino N decreased; further study is, however, needed to confirm this. The lower protein accretion in period 3 than in period 1 was caused by protein breakdown being higher in period 3. Both protein synthesis and breakdown were lowest in period 5, and this could be expected because the animals had reached mature body size by then. The values for synthesis and breakdown in these almost mature minks were higher than those observed in mature cats (Russell *et al*. 2003). Although both the mink and the cat are strict carnivores, there might be species differences in the rate of protein synthesis and breakdown. Other reasons for the discrepancies may be that the use of 24 h as the cut-off time may have resulted in overestimation of the protein synthesis and breakdown in the minks. Furthermore, the cat data (Russell *et al*. 2003) were calculated based on label excreted in urea and ammonia separately, whereas our data were calculated based on total urinary N.

The protein synthesis, breakdown, and flux in pigs as found in this experiment were in the higher range of the values reported by Roth *et al*. (1999), Gotterbarm *et al*. (1998), Windisch *et al*. (2000), and Saggau *et al*. (2000). The higher values we found were probably an effect of higher protein intake, because the protein intake was lower in the experiments by Saggau *et al*. (2000), though high enough to cover the requirement. Reeds *et al*. (1980, 1981) and Fuller *et al*. (1987) have shown that higher protein intake can cause higher protein synthesis and breakdown. The lysine

content in the starter diet decreased with increasing dietary BPM content. This probably caused some negative effects, which are discussed in Hellwing *et al*. (2005*c*). The data indicated that protein synthesis and breakdown were lowest on P4 in period 2, and although the differences were non-significant; this finding might be attributed to the lysine content of this diet being lower than required. A supply of lysine below the requirement has been shown to decrease the protein flux, synthesis, breakdown, and retention (Salter *et al*. 1990). Calculated in relation to metabolic body size, protein synthesis decreased with age, but if calculated in g of protein per day, synthesis and breakdown *increased* significantly, which is in agreement with Reeds *et al*. (1980).

Protein synthesis, breakdown, and flux were considerably higher in the pigs than in the minks. If a higher protein intake caused a higher protein synthesis and breakdown (pigs: Reeds *et al*. 1980, 1981; Fuller *et al*. 1987; cats: Russell *et al*. 2003), the higher IN measured for the pigs may offer an explanation. Furthermore, modern pig breeds have been selected for a high rate of protein accretion, which is not the case in the mink.

Breath testing

The cumulative excretion of $^{13}CO_2$ was affected by period but not diet. Tauson *et al.* (2000) could not demonstrate any differences in the decarboxylation of 1-^{13}C-leucine between minks fed diets based on fish meal and those fed diets based on soybean meal. In lactating mink dams, the level of protein supply has been shown to influence the oxidation rate (Tauson *et al.* 2001*a*). The higher cumulative excretion found in period 3 (late August–early September) than in period 5 (mid November) was contrary to the findings of Børsting and Riis (2000), who found a higher oxidation of uniformly labelled leucine in minks in August than in November. The difference in choice of label may, however, have played a role in this difference. Glycine is a glycogenic amino acid, which can be recycled to glucose whereas leucine is ketogenic; furthermore, Børsting and Riis (2000) used a uniformly labelled amino acid, whereas the glycine in our study was only labelled at the carboxyl atom.

Nucleic acid and creatinine metabolism

The NAN intake did not increase on M4 compared with M3, due to the lower feed intake on M4. The decrease in thymine with increasing dietary content of BPM was unexpected, but as thymine only appears in DNA, the relative content of DNA may be higher in the fish meal used here than in the BPM (Herbel & Montag, 1987).

The 24-h urinary creatinine excretion differed between the pigs and the minks. The pigs excreted more creatinine in relation to metabolic body size than the minks did, and this can possibly be related to the greater muscle mass of the pig. In the minks, the excretion decreased from 0.83 mmol/kg$^{0.75}$ on M1 to 0.21 mmol/kg$^{0.75}$ on M4, while the level on M2 and M3 was the same as that observed by Tauson *et al*. (1997, 2001*b*) in adult female minks. In pigs, no differences in the excretion of creatinine between diets were found for either 24-h excretion or for concentration in urine. The creatinine excretion may be used as a non-specific measure of renal function. In rats, a decreased urinary concentration of creatinine and increased urine production have been observed when the rats were fed free adenine (Yokozawa *et al*. 1982, 1983; Brúle *et al*. 1988). In the mink experiment, the urine volume decreased with increasing dietary BPM content (Hellwing *et al.* 2005*a*), indicating that the renal function was not impaired and that the content of free adenine from the diet or from the intestinal decomposition of RNA and DNA did not reach the level where problems have been observed (Clifford & Story, 1976). The decrease in creatinine excretion with increasing dietary BPM in the minks cannot be explained from the experimental data. However, because arginine is the substrate for creatinine synthesis, it may be speculated that this amino acid, which is of fundamental importance in the urea cycle of strict carnivores (Morris, 1985), might have become increasingly limiting as the intake of amino acids decreased.

The metabolism of the pyrimidine bases is difficult to follow, as they are decomposed into products that can be completely oxidised. In both the pigs and minks, the excretion of allantoin increased with increasing dietary intake of purine bases; in pigs, uric acid and xanthine excretion increased as well, whereas xanthine excretion tended to decrease in the minks. In both species, allantoin made up more than 90% of the excreted purine base derivatives, and the influence of the other derivatives on the total excretion was negligible. Increased excretion of allantoin has also been observed in pigs fed yeast RNA or other types of bacterial protein (D'Mello *et al*. 1976;

Braude *et al*. 1977; Roth & Kirchgessner 1977*a,b*, 1978; Greife *et al*. 1984). The observed species differences in terms of total excretion and the ratio between the different purine base derivatives suggested that the minks examined in this experiment decomposed the purine bases to their end-product allantoin more completely than the pigs did. The observed differences could be a possible effect of the difference in guanine and adenine intake. The content of guanine was lower than that of adenine in the mink diet while the opposite was the case in the pig diets, which might have affected the proportions of different purine base derivatives in the urine. Another explanation for the species differences in these traits might be that the pig has been shown to have a net secretion of urate in the nephron (Weiner, 1979). No corresponding data are available regarding the mink, but the cat is known to have a net reabsorption of urate (Weiner, 1979).

The output of purine derivatives compared to the intake of purine bases differed between species: in both species the ratio decreased, but in the minks more purine base derivatives were excreted than ingested on all diets. Even though the above is a very simplified estimate, ignoring the facts that not all purine bases are digested and that other excretion routes are possible, it is known that other species differ in their utilisation and excretion of dietary purines. From studies in pigs and humans it is known that purine base derivatives are excreted into the gastrointestinal tract (Sørensen, 1960; Greife & Molnar, 1984*b*). Also, the retention of dietary purine bases may differ between species. It has been documented that pigs retained up to 40% and 15% of radioactively labelled adenosine monophosphate (AMP) and guanosine monophosphate (GMP), respectively, 24 h after an oral dose (Greife & Molnar, 1984*b*). In rats, only 5% of the orally given AMP and 2% of the GMP were retained (Greife & Molnar, 1983), and in chickens up to 15% of the orally given AMP and 3% of the GMP were retained (Greife and Molnar, 1984*a*).

The fasting plasma concentrations of creatinine, uric acid, xanthine, and hypoxanthine were not affected by dietary BPM content. It is known that free adenine, but not adenosine, AMP, guanine, guanosine, or GMP, may elevate plasma concentrations of uric acid, creatinine, and allantoin (Yokozawa *et al*. 1982, 1983; Brúle *et al*. 1988). It may therefore be concluded that the diets used in this experiment did not exert any uricogenic effect in the pigs. The plasma concentration of allantoin has been shown to be increased in pigs fed yeast RNA and methanol-grown bacterial protein (Roth & Kirchgessner, 1977*a,b*), but as we did not measure the plasma concentration of allantoin we cannot estimate the impact of dietary BPM on this trait.

Conclusions

The protein turnover rate, but not the net protein synthesis, increased in the minks, but not in the pigs, with increasing dietary BPM. The increased protein turnover in the mink experiment was probably related to the amino acid composition of the diet. Breath testing data from the mink experiment indicated that protein decarboxylation rate was not affected by diet.

The increasing intake of NAN with increasing dietary content of BPM led to the increased excretion of allantoin in both minks and pigs. Furthermore, the data suggested that the metabolism of adenine and guanine was more complete in the mink than in the pig.

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Table 1. Formulation and chemical composition of the experimental mink diets (g/kg), calculated percentage of ME derived from protein, fat, and carbohydrate, and contents of purine and pyrimidine bases (mg/kg).

	M1		M2		M3		M4	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Ingredients								
Bacterial protein meal	0		45		90		135	
Fish meal	120		80		40		0	
Cod fillet	150		150		150		150	
Whole chicken*	120		120		120		120	
Potato mash powder	40		40		40		40	
Heat-treated wheat and barley	80		80		80		80	
Rolled oats	40		40		40		40	
Soy oil	40		40		40		40	
Sugar beet pulp	20		20		20		20	
Vitamins and minerals†	2		2		2		2	
Water	390		385		380		375	
Chemical composition								
Number of analyses‡	6		6		6		6	
Dry matter	394	9.4	394	5.2	396	2.7	402	1.7
Organic matter	365	7.9	370	4.2	375	2.7	384	2.3
Ash	29	1.6	24	1.4	21	0.2	18	1.0
Nitrogen	25	1.2	24	0.8	24	0.4	24	0.4
Protein (N * 6.25)	153		152		150		153	
Fat	77	3.9	77	0.8	76	1.7	76	1.3
Carbohydrate	136	5.5	141	4.1	149	3.2	155	2.7
Gross energy (kJ/kg)	9021	185	9043	177	9183	189	9218	147
Energy distribution: protein:fat:carbohydrate (% of ME)	35:41:24		35:41:24		35:42:23		35:42:23	
Adenine	455		663		952		1209	
Guanine	1810		2066		2345		2539	
Cytosine	348		659		922		1199	
Uracil	113		539		859		1032	
Thymine	360		298		240		198	
% of N from purine and pyrimidine bases	5.8		7.7		9.9		11.2	

* Chicken prepared for human consumption, i.e. without head, feet, feather, and entrails.

† Composition of supplement (content per kg supplement): Vitamins: retinol 2800500 IU, cholecalciferol 280000 IU, α-tocopheryl acetate 24021 IU, thiamine 10002 mg, riboflavin 4801 mg, nicotinamide 8002 mg, pyridoxine 3201 mg, D-pantothenic acid 3207 mg, biotin 80012 µg, folic acid 241 mg, choline chloride 60001 mg, cyanocobalamin 16008 µg, para-aminobenzoic acid 800 mg, betaine 33600 mg; minerals: Fe ($FeSO_4$) 19712 mg, Cu ($CuSO_4$) 1025 mg, Zn (ZnO) 12561 mg, Mn (MnO) 6238 mg. The vitamins and minerals were mixed with calcium carbonate and soy husk meal (Trouw Nutrition Denmark A/S, Vejen, Denmark).

‡ Six small batches of feed were used in the mink experiment. From each batch a sample was analysed. In the table the means are given with the standard deviation.

Table 2. Formulation and chemical composition of the pig experimental diets (g/kg), gross energy (MJ/kg), and purine and pyrimidine bases (mg/kg).

	Starter diet				Growing–finishing diet			
	P1	P2	P3	P4	P1	P2	P3	P4
Ingredients								
Barley	226	246	250	270	487	518	541	563
Wheat	480	480	500	497	250	250	250	250
Bacterial protein meal (BPM)	0	52	101	153	0	50	100	150
Soybean meal (45% crude protein	210	140	68	0	224	146	72	0
Soy oil	40	40	40	40	6	3.6	5.0	5.0
Lime stone	15.8	16.2	16.6	16.9	14.2	14.2	14.2	14.2
Monocalcium phosphate	15.4	14.8	14.4	13.9	11.7	11.7	11.8	11.8
Sodium chloride	4.7	4.6	4.5	4.40	3.8	3.7	3.6	3.5
Iron fumarate	0.33	0.33	0.33	0.33				
Zinc oxide	0.10	0.10	0.10	0.10				
Vitamins and minerals*	3.03	3.03	3.03	3.03				
Vitamin and minerals†					1.8	1.8	1.8	1.8
L-lysine HCl (98%)	3.4	2.5	1.8	0.7	0.65	0.64	0.51	0.34
DL-methionine	0.30	0	0	0	0.54	0.32	0.06	0
L-threonine	1	0.63	0.4	0	0.60	0.37	0.09	0
L-tryptophan	0.15	0.21	0.3	0.35				
Chemical composition								
Dry Matter	919	917	918	916	881	886	892	890
Ash	67.4	59.3	59.6	59.0	55.8	54.9	50.5	54.6
Nitrogen	31.7	31.7	32.0	32.9	30.6	31.5	30.3	30.4
Crude protein (N * 6.25)	198	198	200	206	191	197	189	190
Fat	36.9	40.3	52.1	73.5	27	32	40	43
Carbohydrate‡	617	619	606	578	608	603	612	603
Gross energy	17.5	17.4	17.5	17.8	16.0	16.3	16.7	16.6
Adenine	362	831	1265	1766	401	928	1213	1568
Guanine	321	712	1059	1499	333	792	1043	1366
Cytosine	466	868	1125	1663	470	979	1288	1604
Uracil	667	953	1085	1429	711	1145	1360	1558
Thymine	128	192	244	322	114	217	228	255
% of N from purine and pyrimidine bases	2.5	5.0	7.1	9.6	2.7	5.6	7.7	9.7

* Vitamins and trace elements included to provide the following amounts per kg^{-1} of feed: 140 mg of Zn; 201 mg of Fe; 80 mg of Mn; 20 mg of Cu; 10 mg of I, 0.4 mg of Se; 11000 IU of vitamin A; 1375 IU of cholecalciferol; 137.5 mg of d-α-tocopheryl acetate; 6.9 mg of riboflavin; 22.9 mg of d-pantothenic acid; 27.5 μg of cyanocobalamin; 840 mg choline chloride; 600 mg ascorbic acid.

†Vitamins and trace elements included to provide the following amounts per kg of feed: 105 mg Zn; 75 mg Fe; 60 mg Mn; 15 mg Cu; 7.44 mg I; 0.3 mg Se; 8400 IU vitamin A, 700 IU cholecalciferol; 115.9 mg d-1-α-tocopheryl acetate; 5 mg riboflavin; 15 mg d-pantothenic acid; 20 mg cyanocobalamin; 600 mg choline chloride.

‡ Calculated by difference

Table 3. Protein metabolism and turnover in mink kits fed diets in which 0% (M1), 20% (M2), 40% (M3), and 60% (M4) of N was derived from bacterial protein meal.

	Diet				Period						*P* values			
	M1	M2	M3	M4	1	2	3	4	5	RMSE*	Diet (D)	Period (P)	D*P	Block
Age in weeks					9.5	14.5	17.5	23.5	28.5					
Number of observations (n)†	18	18	18	20	14	14	14	16	16					
Body weight (g)	2038^{a}	1938^{ab}	1784^{bc}	1776^{c}	871^{E}	1585^{D}	1912^{C}	2439^{B}	2609^{A}	239	0.003	<0.001	0.99	<0.001
Nitrogen balance data‡														
IN (g/kg$^{0.75}$)	2.82	2.82	2.86	2.48	3.27^{A}	3.23^{A}	3.40^{A}	2.31^{B}	1.51^{C}	0.50	0.07	<0.001	0.90	0.04
FN (g/kg$^{0.75}$)	0.47^{b}	0.54^{ab}	0.61^{a}	0.57^{a}	0.73^{A}	0.66^{AB}	0.64^{B}	0.44^{C}	0.27^{D}	0.11	0.01	<0.001	0.61	0.22
UN (g/kg$^{0.75}$)	1.89^{a}	1.75^{a}	1.73^{a}	1.52^{b}	1.61^{C}	2.00^{B}	2.28^{A}	1.56^{C}	1.15^{D}	0.31	0.01	<0.001	1.00	0.01
RN (g/kg$^{0.75}$)	0.45	0.54	0.52	0.40	0.94^{A}	0.56^{B}	0.48^{B}	0.32^{C}	0.09^{D}	0.18	0.06	<0.001	0.02	0.47
Protein turnover§														
I (g N/kg$^{0.75}$)	2.56^{a}	2.51^{a}	2.66^{a}	2.05^{b}	3.00^{A}	2.90^{A}	2.97^{A}	2.16^{B}	1.19^{C}	0.51	0.002	<0.001	0.33	0.36
E (g N/kg$^{0.75}$)	1.70^{ab}	1.78^{a}	1.82^{a}	1.47^{b}	1.61^{B}	1.95^{A}	2.13^{A}	1.64^{B}	1.14^{C}	0.38	0.03	<0.001	0.81	0.01
S (g protein/kg$^{0.75}$)	15.0^{b}	14.8^{b}	17.6^{ab}	20.3^{a}	21.9^{A}	16.2^{B}	21.4^{A}	13.0^{B}	12.2^{B}	5.3	0.01	<0.001	0.55	0.06
B (g protein/kg$^{0.75}$)	9.7^{b}	10.4^{b}	12.6^{b}	16.8^{a}	13.2^{AB}	10.3^{B}	16.1^{A}	10.2^{B}	12.1^{B}	5.3	0.001	0.03	0.80	0.21
Flux rate (g protein/kg$^{0.75}$)	25.6	25.9	29.0	29.5	32.0^{AB}	28.4^{B}	34.7^{A}	23.3^{C}	19.3^{C}	5.5	0.08	<0.001	0.34	0.52
Net protein synthesis (g/kg$^{0.75}$)	5.2	4.4	5.0	3.5	8.7^{A}	5.9^{B}	5.3^{B}	2.8^{C}	0.1^{D}	2.2	0.08	<0.001	0.08	0.11

IN, intake of N; FN, faecal N; UN, urinary N; RN, retained N; I, nitrogen intake; E, nitrogen excretion; S, synthesis; B, breakdown

* Root mean square error

† Six observations were omitted because of technical problems.

‡ Average of four days

§ Protein turnover measured by means of end-product methods using [^{15}N]glycine as the tracer; intake and excretion of nitrogen are 24-h measurements.

^{a,b,c} Values with different superscripts differ significantly, effect of diet ($P < 0.05$).

^{A,B,C,D} Values with different superscripts differ significantly, effect of period ($P < 0.05$).

Table 4. Protein metabolism and turnover in castrated male pigs fed diets in which 0% (P1) and up to 17% (P2), 35% (P3) and 52% (P4) of the N was derived from bacterial protein meal.

	Diet				Period				RR[*]	*P* values			
	P1	P2	P3	P4	1	2	3	4		Diet (D)	Period (P)	D*P	Block
Weight of animals[‖]					10.1	21.7	47.5	79.1					
n[†]	15/7	16/8	16/8	16/8	16/0	15/15	16/0	16/16					
Body weight (kg)	49.0	51.4	50.5	51.2	10.1^{D}	21.7^{C}	47.5^{B}	79.1^{A}	0.43	0.64	<0.001	0.93	<0.001
Nitrogen balance data[‡]													
IN (g/kg$^{0.75}$)	3.21	3.40	3.18	3.38	2.33^{D}	3.76^{B}	4.07^{A}	3.03^{C}	0.14	0.15	<0.001	0.07	<0.001
FN (g/kg$^{0.75}$)	0.73	0.82	0.84	0.86	0.62^{B}	0.98^{A}	1.01^{A}	0.64^{B}	0.002	0.12	<0.001	0.13	0.14
UN (g/kg$^{0.75}$)	0.99	1.06	1.01	1.06	0.67^{C}	0.97^{B}	1.26^{A}	1.22^{A}	0.09	0.44	<0.001	<0.001	0.12
RN (g/kg$^{0.75}$)	1.50	1.53	1.33	1.46	1.04^{B}	1.81^{A}	1.80^{A}	1.16^{B}	0.13	0.08	<0.001	0.53	<.001
Protein turnover[§]													
I (g N/kg$^{0.75}$)	2.72	2.63	2.82	2.82		2.81		2.68	0.13	0.30	0.09	0.04	0.05
E (g N/kg$^{0.75}$)	1.09	1.07	1.15	1.12		0.92^{B}		1.30^{A}	0.10	0.84	<0.001	<0.001	0.39
S (g protein/kg$^{0.75}$)	34.0	31.7	32.7	30.8		36.0^{A}		28.6^{B}	1.00	0.91	0.03	0.32	0.23
B (g protein/kg$^{0.75}$)	23.8	21.9	22.2	20.2		24.2		19.9	1.00	0.88	0.19	0.31	0.09
Flux rate (g protein/kg$^{0.75}$)	40.9	38.4	39.8	37.8		41.8		36.7	1.00	0.92	0.10	0.46	0.21
Net protein synthesis (g/kg$^{0.75}$)	10.1	9.8	10.4	10.5		11.8^{A}		8.7^{B}	0.78	0.65	<0.001	0.41	0.01

IN, intake of N; FN, faecal N; UN, urinary N; RN, retained N; I, nitrogen intake; E, nitrogen excretion; S, synthesis; B, breakdown

[*] Residual error

[†] Number of observations in balance experiment/protein turnover experiment; one pig was injured during the second balance period and its results were omitted.

[‡] Data for the nitrogen balance regards data from the four balance periods

[‖] Average weight of the pigs during the balance period

[§] Protein turnover measured end-product methods using [^{15}N]glycine as the tracer; intake and excretion of nitrogen are 48-h measurements

[A,B,C,D] Values with different superscripts differ significantly; effect of period (P<0.05).

Table 5. Metabolism of creatinine and purine bases in mink kits fed diets in which 0% (M1), 20% (M2), 40% (M3), and 60% (M4) of N was derived from bacterial protein meal.

	Diet				Period						*P* values			
	M1	M2	M3	M4	1	2	3	4	5	RMSE*	Diet (D)	Period (P)	D*P	Block
Age in weeks					9.5	14.5	17.5	23.5	28.5					
Number of observations (n) †	18	18	20	20	14	14	16	16	16					
Intake														
Feed intake (g/kg$^{0.75}$)	117^{a}	117^{a}	122^{a}	104^{b}	143^{A}	136^{A}	136^{A}	95^{B}	64^{C}	19.5	0.03	<0.001	0.71	0.04
Adenine (g/kg$^{0.75}$)	0.05^{c}	0.08^{b}	0.11^{a}	0.12^{a}	0.11^{A}	0.11^{A}	0.11^{A}	0.08^{B}	0.05^{C}	0.02	<0.001	<0.001	0.01	0.04
Guanine (g/kg$^{0.75}$)	0.21^{c}	0.24^{bc}	0.28^{a}	0.26^{b}	0.30^{A}	0.29^{A}	0.30^{A}	0.20^{B}	0.13^{C}	0.05	<0.001	<0.001	0.413	0.03
Cytosine (g/kg$^{0.75}$)	0.04^{d}	0.08^{c}	0.11^{b}	0.12^{a}	0.10^{A}	0.11^{A}	0.11^{A}	0.07^{B}	0.05^{C}	0.02	<0.001	<0.001	0.004	0.04
Uracil (g/kg$^{0.75}$)	0.01^{c}	0.06^{b}	0.10^{a}	0.10^{a}	0.08^{A}	0.09^{A}	0.09^{A}	0.06^{B}	0.04^{C}	0.02	<0.001	<0.001	<0.001	0.05
Thymine (g/kg$^{0.75}$)	0.04^{a}	0.03^{b}	0.03^{c}	0.02^{d}	0.03^{A}	0.03^{A}	0.03^{A}	0.02^{B}	0.02^{C}	0.01	<0.001	<0.001	0.59	0.09
NAN (g/kg$^{0.75}$)	0.15^{c}	0.20^{b}	0.26^{a}	0.26^{a}	0.26^{A}	0.26^{A}	0.27^{A}	0.18^{B}	0.12^{C}	0.04	<0.001	<0.001	0.11	0.03
Excreted														
Creatinine (mmol/kg$^{0.75}$)	0.83^{a}	0.59^{b}	0.39^{c}	0.21^{d}	0.41^{C}	0.55^{B}	0.65^{A}	0.53^{BC}	0.38^{D}	0.09	<0.001	<0.001	0.01	0.02
Allantoin (mmol/kg$^{0.75}$)	1.50^{b}	1.71^{ab}	1.86^{a}	1.87^{a}	1.58^{C}	2.02^{AB}	2.26^{A}	1.77^{BC}	1.03^{D}	0.39	0.02	<0.001	0.16	0.04
Uric acid (µmol/kg$^{0.75}$)	72.6	70.9	69.4	64.6	67.7^{BC}	83.7^{B}	119.9^{A}	46.5^{CD}	29.0^{D}	35.2	0.91	<0.001	1.00	0.07
Xanthine (µmol/kg$^{0.75}$)	6.47^{a}	3.78^{b}	1.69^{b}	3.34^{b}	5.66	4.46	4.74	2.87	1.38	4.41	0.02	0.08	0.98	0.58
Hypoxanthine (µmol/kg$^{0.75}$)	2.13^{a}	1.91^{a}	1.99^{a}	1.41^{b}	2.20^{AB}	2.39^{A}	1.76^{B}	1.78^{B}	1.17^{C}	0.62	0.004	<0.001	0.58	0.14
Purine bases out/in	1.54^{a}	1.43^{a}	1.21^{b}	1.21^{b}	0.57^{D}	1.10^{C}	1.39^{B}	1.89^{A}	1.78^{A}	0.21	<0.001	<0.001	0.81	0.01
% of urinary N														
Creatinine (%)	1.96^{a}	1.54^{b}	1.06^{c}	0.69^{d}	1.14^{C}	1.19^{BC}	1.24^{B}	1.45^{A}	1.54^{A}	0.14	<0.001	<0.001	0.27	0.54
Total purines (%)	4.97^{d}	6.13^{c}	6.80^{b}	7.49^{a}	6.31^{B}	6.29^{B}	6.30^{B}	6.98^{A}	5.86^{B}	0.71	<0.001	0.001	<0.001	0.74
Others (%)	93.07^{a}	92.33^{b}	92.14^{bc}	91.82^{c}	92.55^{A}	92.53^{A}	92.46^{A}	91.56^{B}	92.60^{A}	0.72	<0.001	0.001	<0.001	0.84
Individual purine base derivatives % of total purine base derivatives														
Allantoin (%)	95.02^{b}	95.98^{a}	96.41^{a}	96.79^{a}	95.73^{B}	95.59^{BC}	94.62^{C}	97.23^{A}	97.09^{A}	1.36	0.002	<0.001	0.96	0.07
Uric acid (%)	4.46^{a}	3.72^{ab}	3.39^{b}	2.94^{b}	3.81^{B}	4.07^{B}	5.09^{A}	2.51^{C}	2.67^{C}	1.33	0.01	<0.001	0.95	0.05
Xanthine (%)	0.38^{a}	0.20^{b}	0.09^{b}	0.16^{b}	0.30	0.22	0.22	0.16	0.13	0.22	0.002	0.32	0.99	0.95
Hypoxanthine (%)	0.14	0.11	0.11	0.10	0.16^{A}	0.12^{AB}	0.08^{B}	0.10^{B}	0.11^{AB}	0.07	0.33	0.04	0.73	0.06

NAN, nucleic acid nitrogen

* root mean square error

† Six observations were omitted because of technical problems.

^{a,b,c} Values with different superscripts differ significantly; effect of diet ($P < 0.05$).

^{A,B,C,D} Values with different superscripts differ significantly; effect of period ($P < 0.05$).

Table 6. Metabolism of creatinine and purine bases in castrated male pigs fed diets in which 0% (P1) and up to 17% (P2), 35% (P3), and 52% (P4) of the N was derived from bacterial protein meal.

	Diet				Period				RR*	*P* values			
	P1	P2	P3	P4	1	2	3	4		Diet (D)	Period (P)	D*P	Block
Weight (Kg)†					10.1	21.7	47.5	79.1					
Number of pigs (n)‡	15	16	16	16	16	15	16	16					
Intake													
DM (g/kg$^{0.75}$)	93	97	93	97	67^{D}	107^{B}	118^{A}	88^{C}	0.95	0.38	<0.001	0.26	<0.001
Adenine (g/kg$^{0.75}$)	0.04^{d}	0.09^{c}	0.13^{b}	0.18^{a}	0.08^{D}	0.12^{B}	0.14^{A}	0.10^{C}	0.004	<0.001	<0.001	<0.001	<0.001
Guanine (g/kg$^{0.75}$)	0.05^{d}	0.14^{c}	0.19^{b}	0.27^{a}	0.11^{D}	0.18^{B}	0.20^{A}	0.15^{C}	0.01	<0.001	<0.001	<0.001	0.002
Cytosine (g/kg$^{0.75}$)	0.05^{d}	0.10^{c}	0.12^{b}	0.17^{a}	0.08^{D}	0.12^{B}	0.14^{A}	0.11^{C}	0.0001	<0.001	<0.001	<0.001	0.001
Uracil (g/kg$^{0.75}$)	0.07^{d}	0.11^{c}	0.13^{b}	0.16^{a}	0.08^{D}	0.12^{B}	0.16^{A}	0.12^{C}	0.01	<0.001	<0.001	0.01	<0.001
Thymine (g/kg$^{0.75}$)	0.01^{c}	0.02^{b}	0.02^{b}	0.03^{a}	0.02^{C}	0.03^{A}	0.03^{A}	0.02^{B}	0.002	<0.001	<0.001	<0.001	<0.001
NAN (g/kg$^{0.75}$)	0.08^{d}	0.18^{c}	0.24^{b}	0.33^{a}	0.14^{D}	0.23^{B}	0.26^{A}	0.19^{C}	0.01	<0.001	<0.001	<0.001	<0.001
Excreted													
Creatinine (mmol/kg$^{0.75}$)	0.62	0.71	0.72	0.70	0.40^{C}	0.44^{C}	0.83^{B}	1.08^{A}	0.06	0.25	<0.001	0.84	0.005
Allantoin (mmol/kg$^{0.75}$)	0.77^{b}	1.00^{b}	0.97^{b}	1.08^{a}	0.53^{C}	1.00^{B}	1.33^{A}	0.98^{B}	0.15	0.002	<0.001	0.25	0.50
Uric acid (µmol/kg$^{0.75}$)	14.8^{b}	20.8^{b}	31.9^{a}	38.1^{a}	32.6^{A}	27.9^{AB}	25.2^{B}	19.9^{B}	0.84	<0.001	<0.001	0.04	0.18
Xanthine (µmol/kg$^{0.75}$)	13.2^{c}	19.3^{b}	25.0^{a}	26.7^{a}	13.0^{C}	24.4^{A}	25.3^{A}	21.5^{B}	0.81	<0.001	<0.001	0.03	0.42
Hypoxanthine (µmol/kg$^{0.75}$)	10.7	21.2	18.5	41.1	3.3^{C}	16.3^{B}	32.0^{A}	40.0^{A}	0.96	0.10	0.001	0.42	0.75
Purine bases out/in	1.26^{a}	0.66^{b}	0.48^{c}	0.38^{c}	0.56^{B}	0.71^{A}	0.76^{A}	0.75^{A}	0.09	<0.001	<0.001	0.02	0.22
% of urinary N													
Creatinine (%)	2.8	2.9	3.1	3.0	3.0^{B}	2.1^{C}	2.9^{B}	3.9^{A}	0.15	0.71	<0.001	0.09	0.16
Total purines (%)	5.0^{b}	6.1^{a}	6.1^{a}	6.5^{a}	5.6^{B}	6.5^{A}	6.5^{A}	5.1^{B}	0.53	0.002	<0.001	0.02	0.71
Others (%)	92.2^{a}	91.0^{b}	90.8^{b}	90.4^{b}	91.4	91.4	90.6	91.0	0.38	0.02	0.06	0.05	0.16
Individual purine base derivatives % of total purine base derivatives													
Allantoin (%)	94.8^{a}	93.9^{ab}	92.4^{bc}	90.8^{c}	91.3^{C}	93.8^{AB}	94.3^{A}	92.5^{BC}	0.04	0.008	<0.001	0.83	0.13
Uric acid (%)	2.2^{c}	2.5^{bc}	3.5^{ab}	3.8^{a}	5.8^{A}	2.5^{B}	1.8^{C}	1.9^{C}	0.05	0.02	<0.001	0.001	0.19
Xanthine (%)	1.7^{b}	1.9^{b}	2.4^{a}	2.4^{a}	2.3^{A}	2.3^{A}	1.8^{B}	2.0^{A}	0.27	<0.001	<0.001	0.36	0.07
Hypoxanthine (%)	1.4	1.7	1.6	3.0	0.6^{D}	1.4^{C}	2.2^{B}	3.6^{A}	0.20	0.22	0.001	0.65	0.19
Plasma concentrations													
Creatinine (mmol/ml)	82.1	87.8	85.6	87.5	84.5^{B}	73.4^{C}	70.4^{C}	114.7^{A}	0.99	0.51	<.001	0.38	0.82
Uric acid (mmol/ml)	68.0	65.8	65.2	55.3	61.0^{B}	52.5^{B}	70.4^{A}	70.4^{A}	0.98	0.08	<.001	0.46	0.29
Xanthine (mmol/ml)	9.4	11.1	11.2	12.3	3.5^{C}	5.2^{B}	13.7^{A}	21.6^{A}	1.27	0.72	<.001	0.72	0.31
Hypoxanthine (mmol/ml)	15.2	20.3	19.4	16.8	22.8^{AB}	29.2^{A}	15.3^{B}	4.5^{C}	0.77	0.71	<.001	0.74	0.05

DM, dry matter; NAN, nucleic acid nitrogen

* Residual error

† Number of observations in balance experiment; one pig was injured during the second balance period and its results were omitted.

‡ Average weight of the pigs during the balance period

^{a,b,c,d} Values with different superscripts differ significantly; effect of diet ($P < 0.05$).

^{A,B,C,D} Values with different superscripts differ significantly; effect of period ($P < 0.05$).

Table 7. Comparison of selected traits of the N metabolism, protein turnover, purine base, and creatinine metabolism in pigs and mink fed diets in which up to 0% (D1), 20% (D2), 40% (D4), and 60% (D6) of the N was derived from bacterial protein meal.

	Diet				Species			*P* values		
	D1	D2	D3	D4	Pig	Mink	RMSE*	Diet	Species	Diet*Species
Relative weight gain	1.38	1.38	1.38	0.83	2.42^{A}	0.07^{B}	1.11	0.10	<0.001	0.006
DM (g/kg$^{0.75}$)	68.4	70.6	69.7	68.7	94.8	43.8	18.3	0.96	<0.001	0.65
N metabolism, balance data										
IN (g/kg$^{0.75}$)	2.98	3.07	3.02	2.93	3.29^{A}	2.71^{B}	0.83	0.91	<0.001	0.49
DN (g/kg$^{0.75}$)	2.40	2.43	2.32	2.24	2.51^{A}	2.17^{B}	0.64	0.60	0.002	0.34
FN (g/kg$^{0.75}$)	0.59	0.65	0.70	0.69	0.78^{A}	0.53^{B}	0.22	0.10	<0.001	0.93
UN (g/kg$^{0.75}$)	1.43	1.39	1.37	1.29	1.03^{B}	1.71^{A}	0.42	0.53	<0.001	0.24
RN (g/kg$^{0.75}$)	0.95	1.02	0.93	0.93	1.45^{A}	0.46^{B}	0.38	0.73	<0.001	0.42
ADN (g/kg$^{0.75}$)	80.8^{a}	79.3^{a}	77.1^{b}	76.5^{c}	76.4^{B}	80.4^{A}	2.99	<0.001	<0.001	0.04
Protein turnover, end-product methods using [^{15}N]glycine as tracer										
I (g N/kg$^{0.75}$)	2.63	2.62	2.64	2.43	2.75^{A}	2.41^{B}	0.77	0.77	0.04	0.40
E (g N/kg$^{0.75}$)	1.41	1.44	1.43	1.31	1.12^{B}	1.67^{A}	0.47	0.78	<0.001	0.54
S (g protein/kg$^{0.75}$)	23.8	22.7	24.5	26.5	31.9^{A}	16.8^{B}	7.7	0.42	<0.001	0.73
B (g protein/kg$^{0.75}$)	16.2	15.4	17.0	19.5	21.7^{A}	12.4^{B}	6.8	0.21	<0.001	0.39
Flux rate (g protein/kg$^{0.75}$)	32.6	31.7	33.4	34.7	38.9^{A}	27.2^{B}	8.4	0.68	<0.001	0.85
Net protein synthesis (g/kg$^{0.75}$)	7.5	7.3	7.4	7.0	10.2^{A}	4.4^{B}	3.5	0.95	<0.001	0.64
Purine metabolism										
NAN (g/kg$^{0.75}$)	0.11^{d}	0.19^{c}	0.25^{b}	0.29^{a}	0.21	0.22	0.07	<0.001	0.48	0.001
Excreted										
Creatinine (mmol/kg$^{0.75}$)	0.73^{a}	0.65^{b}	0.55^{bc}	0.46^{c}	0.69^{A}	0.50^{B}	0.24	<0.001	<0.001	<0.001
Allantoin (mmol/kg$^{0.75}$)	1.12^{b}	1.34^{ab}	1.41^{a}	1.47^{a}	0.95^{B}	1.72^{A}	0.50	0.02	<0.001	0.82
Uric acid (µmol/kg$^{0.75}$)	43.2	44.7	50.6	51.4	26.3^{B}	68.6^{A}	34.7	0.69	<0.001	0.29
Xanthine (µmol/kg$^{0.75}$)	9.7^{c}	11.4^{bc}	13.3^{ab}	15.0^{a}	21.1^{A}	4.0^{B}	5.48	0.001	<0.001	<0.001
Hypoxanthine (µmol/kg$^{0.75}$)	6.5^{b}	11.5^{b}	10.3^{b}	21.3^{a}	23.0^{A}	1.8^{B}	17.7	0.01	<0.001	0.003
% of urinary N										
Creatinine (%)	2.40^{a}	2.24^{ab}	2.09^{bc}	1.86^{c}	2.97^{A}	1.32^{B}	0.64	0.004	<0.001	<0.001
Total purine derivatives (%)	4.88^{c}	6.08^{b}	6.44^{b}	7.02^{a}	5.88^{B}	6.33^{A}	1.03	<0.001	0.014	0.17
Others (%)	92.72^{a}	91.67^{b}	91.47^{b}	91.12^{b}	91.14^{B}	92.36^{A}	1.22	<0.001	<0.001	0.73
Individual purine base derivatives % of total purine base derivatives										
Allantoin (%)	94.84	95.00	94.43	93.80	92.95^{B}	96.08^{A}	1.96	0.06	<0.001	<0.001
Uric acid (%)	3.37	3.04	3.44	3.36	3.01^{B}	3.60^{A}	1.78	0.79	0.05	0.004
Xanthine (%)	1.05^{b}	1.03^{b}	1.26^{a}	1.27^{a}	2.10^{A}	0.20^{B}	0.35	0.003	<0.001	<0.001
Hypoxanthine (%)	0.74^{b}	0.92^{ab}	0.88^{b}	1.56^{a}	1.93^{A}	0.11^{B}	1.30	0.04	<0.001	0.03

DM, dry matter; DN, digested N; FN, faecal N; UN, urinary N; RN, retained N; ADN, apparent digestibility of N; I, nitrogen intake; E, nitrogen excretion; S, synthesis; B, breakdown

* Root mean square error ^{a,b,c,d} Values with different superscripts differ significantly; effect of diet ($P < 0.05$). ^{A,B} Values with different superscripts differ significantly; effect of species ($P < 0.05$).

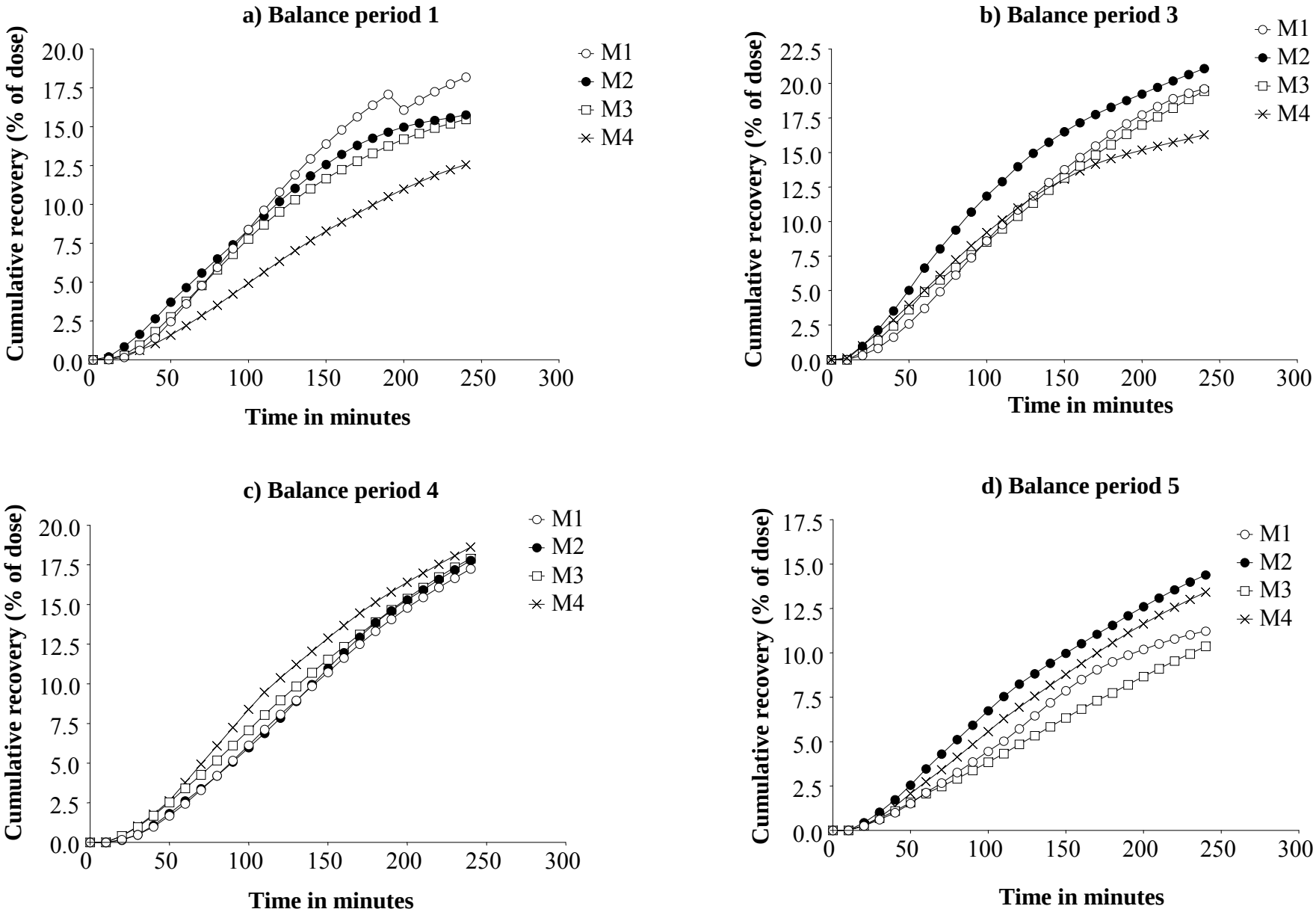

Fig. 1. Cumulative excretion of ^{13}C in periods 1, 3, 4, and 5. *P* values: diet *P*=0.65, period *P*=0.003, time *P*<0.001, diet * period *P*=0.74, diet * time *P*=0.13, period * time *P*<0.001, diet * period * time *P*=0.32, block *P*=0.27

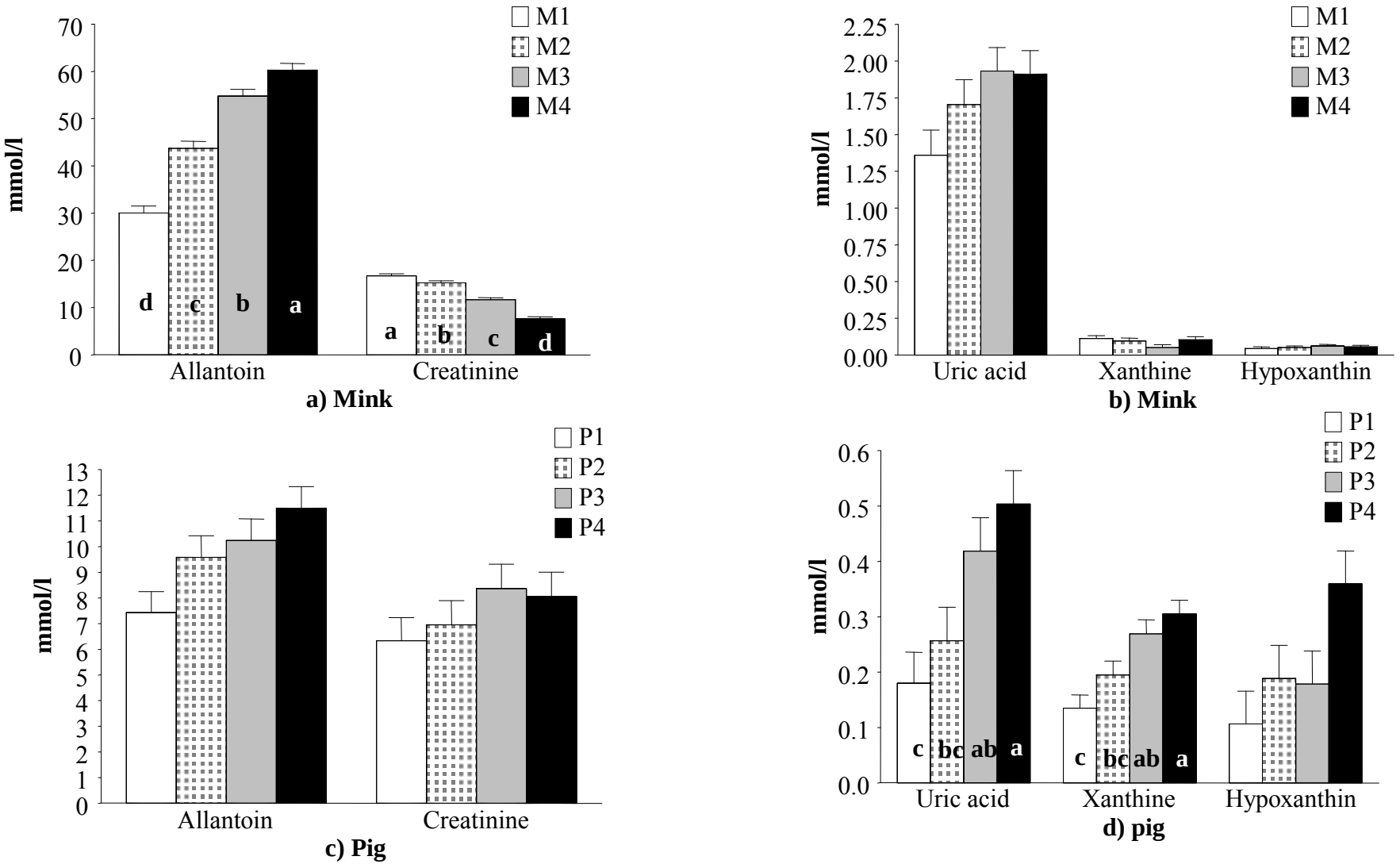

Fig. 2. Urinary concentration of allantoin, creatinine, uric acid, xanthine and hypoxanthine in mink (a and b) fed diets where approximately 0% (M1), 20% (M2), 40% (M3), and 60% (M4) of N was derived from BPM, and in pigs (c and d) fed diets where up to 0% (P1), 17% (P2), 35% (P3), and 52% (P4) of N was derived from BPM. Only the *P* values for the diet effect are given. a: allantoin $P<0.001$, creatinine $P<0.001$; b: uric acid $P=0.07$, xanthine $P=0.08$, and hypoxanthine $P=0.24$; c: allantoin $P=0.07$, creatinine $P=0.42$; d: uric acid $P=0.01$, xanthine $P=0.002$, and hypoxanthine $P=0.06$